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THE UNIVERSITY OF ALBERTA

THE EFFECT OF ANAEROBIC TRAINING ON THE METABOLIC PATTERN OF THE SKELETAL MUSCLE

by

© JEAN JOBIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

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DÉDICATION

A mes parents.

A ma soeur, Louise, et a mon

frère Paul-André; sans leur

support moral et financier tout

ceci ne serait toujours qu'un rêve.

ABSTRACT

Fifty-two male wistar rats (180-260 g) were divided into four groups; sedentary control (C_1, C_2, C_3) exercised (E_1), exercised control (E_2) and trained (T). Exercised, exercised control and trained animals were progressively trained to run at 80 m/min (30% incline) (for two weeks). Exercised animals (E_1) were sacrificed at the end of this training program. Trained animals (T) were continuously trained thereafter for 4 weeks (20 bouts/day, 80 m/min, 30% incline). Exercised control animals were run only once a week (5-8 bouts) to maintain the ability to perform sprint running. Both groups (T and E_2) were given a performance test at the end of the training program, and were sacrificed 24 hours after a regular session. Muscle enzymes (HK, LDH, β-HOADH and T-ICDH) were assayed at physiological pH and T^{o}. Training decreased the effect of growth on the energy supplying metabolism of the skeletal muscle (gastrocnemius). Training also caused an important shift in the metabolic pattern of the muscle toward high glucose phosphorylation. Only HK activity increased significantly ($p<0.05$, 94%) after training. Hexokinase activity was correlated significantly with anaerobic performance ($r= 0.48$, $p<0.05$). Trained rats ran 147% longer than untrained animals at 80 m/min (30% incline). The importance of hexokinase in the energy supplying metabolism after anaerobic work is discussed.

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CHAPTER I

INTRODUCTION

The use of enzyme activities to evaluate the metabolic capacity of tissues is of great value, as they determine the rate of the metabolic pathways where they are involved. However enzyme studies do not have much physiological meaning when individual enzymes are considered; the conditions in which one assays them is very artificial and does not meet physiological conditions (pH, temperature and substrate concentrations). Schmidt and Schmidt (1969) claimed that the only way of keeping enzyme analysis in a physiological context is to express them as ratios of enzymes within a given metabolic pathway or as ratios between enzymes representative of different energy supplying metabolisms.

Pette (1971) as well as Bass et al. (1969) used enzyme ratios to differentiate muscle types. These ratios enable one to evaluate the correlation or interrelation between the different pathways and then to evaluate the physiological implications of one or a group of enzymes in the energy supply to the muscle considered. Thus the use of proportions of enzyme activities enables one to identify the characteristic metabolic pattern of the tissue analysed.

Because of the recognised importance of enzymes as representative of metabolic rates and capacities, exercise physiologists rapidly developed an interest in this area in order to

understand how energy is supplied to the exercising muscle (Gollnick et al., 1973; Edgerton et al., 1972; Baldwin et al., 1972; Staudte et al., 1973). Histochemistry of single enzyme activities is also widely used to determine the precise fiber type distribution of muscles and their modifications under exercise stress (Edgerton et al., 1972; Faulkner et al., 1971; Gollnick et al., 1973).

Holloszy et al. (1967), Gollnick et al. (1973), Staudte et al. (1973) and Exner et al. (1973) have shown modifications of single enzyme activities after different training programs (aerobic, anaerobic, isometric) in humans as well as in laboratory animals. The problem is that, in most of these studies, individual enzyme activities are considered and the difficulty of physiological interpretation is enhanced as the assays do not represent physiological conditions. Furthermore, comparisons are often difficult or impossible to make because of the differences in the experimental conditions (training programs and type of muscle assayed), as stated by Schmidt and Schmidt (1969).

It has been shown by Saubert et al. (1973) and Staudte et al. (1973) that anaerobic training increases the activity of some enzymes in the skeletal muscle of rats. However the training program was quite short in the work of Staudte et al. (1973). Furthermore the study of Saubert et al. (1973) considered glycolytic enzymes only. A more complete study of the energy supplying metabolism is necessary to evaluate the effect of anaerobic training and the relationship between metabolic adaptations and the capacity to perform anaerobic work. In addition, growth is very rarely

considered in studies on exercise, even though the animals used in these studies are growing animals. Wilkinson et al. (1975) as well as Maxwell et al. (1973) have shown that training interacts with the histochemical changes taking place during growth in the muscle of sedentary animals.

The present study was undertaken to continue the work initiated in the area of anaerobic exercise. It was designed to study more closely the energy supplying metabolism of the gastrocnemius muscle of the rats and its adaptation to repeated anaerobic work. Furthermore, this study was designed to take into account the effect of growth on the muscle metabolism as well as the interaction between training and growth.

A number of enzymes were identified as markers of pathways of the energy supplying metabolism and were used to evaluate the capacity of these pathways. The pathways of glucose phosphorylation, lactate fermentation, β-oxidation of fatty acids and the citric acid cycle were selected. The enzymes hexokinase, lactate dehydrogenase, β-hydroxyacyl CoA dehydrogenase and NADP-Specific Isocitrate dehydrogenase were chosen to represent these selected metabolic pathways. Enzyme assays were performed under physiological conditions of pH and temperature to reproduce normal conditions in the muscle.

Another purpose of this study was to evaluate the relationship between the different metabolic pathways selected. The relationships between pathways were measured by the ratios of the enzyme activities. This was carried out in order to estimate the effect of growth and

anaerobic exercise on the metabolic pattern of the skeletal muscle. The following relationships were studied and used to identify shifts in the energy supplying metabolism of the muscle:

Lactate fermentation / citric acid cycle

Lactate fermentation / Glucose phosphorylation

Glucose phosphorylation / citric acid cycle

β-oxidation of fatty acids / citric acid cycle.

Statement of the Problem

This study was designed to determine the effects of anaerobic training on the metabolic pattern of the gastrocnemius muscle of growing rats. Enzyme activities were used to measure the metabolic capacity of lactate fermentation, glucose phosphorylation, the citric acid cycle and the β-oxidation of fatty acids under physiological conditions of pH and temperature.

Limitations

Extrapolations to human beings as well as to other species and other muscle groups must be done with reserve, bearing in mind the differences between species for factors such as the fiber type distribution of the muscle and the metabolic pattern of the muscle studied.

The data on fiber typing (intensity of staining for ATPase activity, α-GPDH activity and NADH-diaphorase activity) was evaluated subjectively using an arbitrary scale.

Definition of Terms and Abbreviations

The classification of muscle fibers will be made on the basis of the observations of Peter et al. (1972):

Fast Glycolytic (FG) - Muscle fibers with high glycolytic capacity, low oxidative capacity, high ATPase activity and fast twitch contractile properties (Low NADH-diaphorase; High ATPase; High α-GPDH).

Slow Oxidative (SO) - Muscle fibers with low glycolytic capacity, high oxidative capacity, low ATPase activity and slow twitch contractile properties (High NADH-diaphorase; Low ATPase; Low α-GPDH).

Fast Glycolytic Oxidative (FOG) - Muscle fibers having high glycolytic capacity, high oxidative capacity, high ATPase activity and fast twitch contractile properties (High NADH-diaphorase; High ATPase; High α-GPDH).

Anaerobic training - training by repetition of anaerobic exercises: high intensity, short duration. Intensity higher than MVO_2; speed 49.5 m/min. in rats (Shepherd and Gollnick, 1976).

Exercised animals - group of animals submitted to two weeks of progressive training in preparation for anaerobic training (E_1).

Exercised control animals - the group of animals submitted to two weeks of progressive training in preparation for anaerobic training; and trained only once a week thereafter for four weeks (E_2).

Sedentary control animals - group of animals handled every day, put on the treadmill as for training but not exercised (C_1, C_2, C_3). The Sedentary Control animals were force confined to their cages (10" x 8" x 6").

Trained animals - group of animals submitted to anaerobic training for four weeks after two weeks of progressive training (T).

Adenosine Triphosphatase (ATPase) Activity - denoted by the intensity of staining procedures and used as an indicator of contractile speed of the muscle fibers (Guth and Samaha, 1969).

Reduced Nicotinamide Adenine Dinucleotide Diaphorase (NADH-diaphorase) Activity - denoted by intensity of staining and used to indicate oxidative capacity of the muscle fibers (Dubowitz and Brook, 1973).

α-Glycerolphosphate dehydrogenase (α-GPDH) activity - denoted by intensity of staining and used to indicate glycolytic capacity of the muscle fibers (Wattenberg and Leong, 1960) (α-GPDH).

Enzyme Activity - activity of the enzyme under physiological conditions (pH 7, 37°C) measured as the amount of substrate transformed per unit of time per gramme of wet muscle (μ moles x min^{-1} x g^{-1} w.w.).

Abbreviations:

α-GPDH: α-Glycerolphosphate dehydrogenase (E.C. 1.1.99.5)

β-HOADH or HOADH: β-hydroxyacyl CoA dehydrogenase (E.C. 1.1.1.35)

CS: citrate synthase (E.C. 4.1.3.7)

D-ICDH: NAD-specific Isocitrate dehydrogenase (E.C. 1.1.1.41)

GAPDH: Glyceraldehyde phosphate dehydrogenase (E.C. 1.2.1.12)

HK: Hexokinase (E.C. 2.7.1.1)

LDH: Lactate dehydrogenase (E.C. 1.1.1.27)

M-ATPase: Myosin-Adenosine triphosphatase (E.C. 3.6.1.3)

MDH: Malate dehydrogenase (E.C, 1.1.1.37)

NADH-diaphorase: Reduced Nicotinamide Adenine Dinucleotide Diaphorase

PFK: Phosphofructokinase (E.C. 2.7.1.11)

SDH: Succinate dehydrogenase (E.C. 1.3.99.1)

T-ICDH: NADP-specific Isocitrate dehydrogenase (E.C. 1.1.1.42)

TPDH: Triose-phosphate dehydrogenase (E.C. 1.2.1.12)

$M\dot{V}O_2$: Maximal oxygen consumption per minute

CHAPTER II

REVIEW OF THE RELATED LITERATURE

Enzyme Ratios and Muscle Differentiation

Enzymes are catalysts of almost all biochemical reactions involved in the energy supplying metabolism. Their activity is then very important to determine the speed of the metabolic reactions. The different tissues have different metabolic properties as well as some common ones. Enzymes can then be used to identify the different metabolic pathways as they represent these pathways and to differentiate the metabolic characteristics of tissues.

Enzyme ratios, introduced by Pette et al., 1962a, were studied in many tissues and have been used to differentiate as well as to find constant characteristics among them (Pette et al., 1962b; Kingenberg and Pette, 1962; Pette, 1966, 1971; and Bass et al., 1969).

The notion of constant proportion enzymes has been used by Pette et al., 1962a, 1962b, and Kingenberg and Pette, 1962, to demonstrate relationships and interdependencies of different metabolic pathways and the constancy of the organization within a given pathway among different tissues.

Pette et al. (1962a) found a constant relationship between enzymes of the respiratory chain (cytochrome a) and cytochrome c in tissues as different as rat brain, skeletal muscle, liver, heart and locusta flight muscle. The same phenomenon was observed for the

enzymes of the pyruvate oxidation and the metabolic segment of transamination in the mitochondrion. However, it was found that the ratios of isocitrate dehydrogenase, glutamate dehydrogenase and glycerol-1-P oxidase to cytochrome c were characteristic of the different tissues analyzed.

Pette et al., 1962b, found a constant proportion group in the Embden-Meyerhof chain in different tissues as well as between different species. On the other hand, the specific proportions of other enzymes with this group could be used to characterize the different energy producing metabolism of the smooth muscle (beef) and the flight muscle of the locusta (low oxidative-glycolytic and high oxidative-high glycolytic respectively).

Pette (1966), differentiated white (semimembranosis) and red (semitendineus) muscle of the rabbit on the basis of their specific glycerol 3-phosphate dehydrogenase (GPOX)/succinate dehydrogenase (SDH) ratios, all the other mitochondrial enzymes studied being in constant proportion with SDH. However, when glyceraldehyde phosphate dehydrogenase (GAPDH) (extramitochondrial constant proportion group marker of glycolysis) was used as the denominator, they noticed a constant proportion between GAPDH and GPOX and a difference of 25 to 30 between the ratios glycolysis (GAPDH)/ citric acid cycle (SDH) in the two tissues. The constancy of GPOX/GAPDH was interpreted as representative of the compensation of the lack of mitochondria in the white muscle by an increase intramitochondrial glycolytic activity (GPOX).

The comparison of the energy supplying metabolisms can then be seen as an efficient way of differentiating the muscle types. On that basis, Bass et al. (1969) assayed eight enzymes representative of the constant proportion groups of the different metabolic systems of glycolysis, β-oxidation of fatty acids, the citric acid cycle and the respiratory chain. Computing the different ratios of the different systems, the authors found, in all species studied, a constancy in the relationship of the following systems in both muscle types (white and red):

Glycogenolysis - Glycolysis

Glycolysis - lactate fermentation

Glucose phosphorylation - citric acid cycle

Fatty acid oxidation - citric acid cycle

However, the two types were very different (White > Red) when the following ratios were considered:

Glycolysis - fatty acid oxidation

Glycolysis - citric acid cycle

Lactate fermentation - citric acid cycle

Glycogenolysis - glucose phosphorylation

Gluconeogenesis - glucose phosphorylation

For these five ratios, the white muscle appeared to be 55 to 175 fold the red muscle in the rabbit [soleus (red) and Psoas (white)]. It is clear then that the enzyme ratios furnish a valuable means of differentiating muscle types on the basis of their energy supplying metabolism as the difference appears to be very well defined between both types.

In agreement with Bass et al. (1969), Staudte and Pette (1972) obtained significant correlations between some enzyme activities representative of the energy supplying metabolism. Glycogen phosphorylase and triosephosphate dehydrogenase gave a correlation coefficient of 0.85, β-hydroxyacyl-CoA dehydrogenase and citrate synthase, hexokinase and citrate synthase, creatine kinase and triosephosphate dehydrogenase, produced correlation coefficients of 0.85, 0.78 and 0.71 respectively. This study was done on 51 muscle specimens from different mammals and birds. These findings show the relationship of the different metabolic systems in the muscle independently of the species and the type of muscle.

However changes in the characteristic ratios of each muscle type can be modified or altered under physiological conditions. Pette (1971), in a review on the metabolic differentiation of distinct muscle types using enzyme ratios, and from a study where a slow muscle was cross innervated and became more like a faster muscle on the enzymatic organization, concluded:

> ... differentiation of energy supplying metabolism in muscle is thus the consequence of various factors. Ultimately, it may be understood as an optimum adaptation of energy-supply to requirements of muscle function.

Bass et al. (1975) observed modifications in the energy-supplying enzyme pattern with ageing. In old rats (28 - 36 months), fast twitch muscle (EDL) and slow twitch muscle (soleus) undergo enzymatic modifications. The former is characterized by a decrease of the ratios of glycolysis/oxidative metabolism and glycolysis/glucose phosphorylation, showing a lower capacity for anaerobic degradation

of substrates. The soleus muscle, on the other hand, reduced its oxidative capacity as well as its glycolytic capacity. Nevertheless, the metabolic pattern of the soleus stays pretty much the same with an overall decrease in energy supplying capacity. In the soleus a usually constant ratio in mitochondria (MDH/CS) decreases. The authors concluded that there was a clear trend of dedifferentiation with old age, as the soleus and the extensor digitorum longus resemble each other more in terms of enzyme activity pattern with ageing.

Constant proportion enzymes or enzyme activity ratios then appear to be an excellent way of studying metabolic changes in muscle tissue under different physiological conditions. The study reported by Pette (1971) as well as the one by Bass et al. (1975) show that the metabolic pattern of the muscle tissue can undergo modifications to adapt to new physiological requirements.

In the studies reported so far, little was done on human skeletal muscle except for the erector tunci, Bass et al. (1969). Even though we know that humans do not have equivalent fiber type distributions as found in other mammals (Edgerton et al., 1975), we assume that about the same metabolic pattern is present in human skeletal muscle. A study by Bass et al. (1975) demonstrated the difficulty in extrapolating experimental results from laboratory animals to the human subjects, as they found an unusual enzyme pattern in the quadriceps femoris of man. This muscle was characterized by a very high capacity for glucose phosphorylation, a high aerobic regeneration of cytoplasmic dehydrogenated NAD (GPDH),

and a very low anaerobic regeneration (LDH). This muscle is capable of breaking down a very high proportion of carbohydrates (glucose) aerobically, as was indicated by the extremely high ratio HK/LDH (Hexokinase/Lactate dehydrogenase) and the extremely low ratio LDH/CS. This is to be considered when comparing work intensity on lactic acid production. Reardon (1975) obtained very high blood lactate values after high intensity exercise in rats compared to what can be obtained in humans.

Enzyme Ratios and Endurance Training

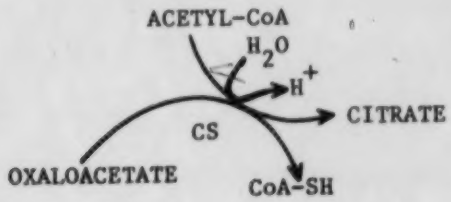
Publications reporting the effect of training on the enzyme ratios of the energy supplying metabolism are scarce. However Bass et al. (1976) as well as Moesch and Howald (1975) reported differences in enzyme ratios between sedentary subjects and endurance trained athletes. Moesch and Howald (1975) measured decreases in the following ratios: HK/SDH, GAPDH/SDH and GAPDH/β-HOADH in trained men. Bass et al. (1976) showed significant decreases in TPDH/CS, HK/CS, GPDS/CS, GPDS/β-HOADH in trained men over sedentary subjects, while LDH/HK was found higher in athletes and β-HOADH/CS was not significantly different in the two groups. These two studies show the relative importance of aerobic metabolism in trained men versus sedentary individuals, as well as an improved capacity to utilize free fatty acids as a source of energy. However these studies are unable to differentiate whether hereditary factors or training was the cause of the particular muscle metabolism of these athletes. It is worth mentioning the differences in ages (means) between the trained and

sedentary subjects in these studies. In Bass et al. (1976), the average age of the athletic group was 24.7 (18-34) years while the sedentary group averaged 39.2 (27-55) years and in Moesch and Howald (1975) the athletes and the sedentary men averaged respectively 23.2 and 33.5 years of age. Thus the differences shown, although they are realistic, are probably over estimated due to the age factor.

Aerobic Training and Enzyme Activity in Skeletal Muscle

Many enzymes of the energy supplying metabolism were shown to undergo changes in optimal activity "in vitro" after an aerobic training program (Holloszy 1967; Kowalski et al., 1969; Baldwin et al., 1972; and Gollnick et al., 1973). Enzymes and co-enzymes of the citric acid cycle and of the respiratory chain were thoroughly studied as they are markers of the oxidative capacity of the muscle cell.

Citrate synthase (CS) and succinate dehydrogenase (SDH) are the most commonly used to measure increased oxidative capacity by the muscle cell. Citrate synthase catalyzes the reaction



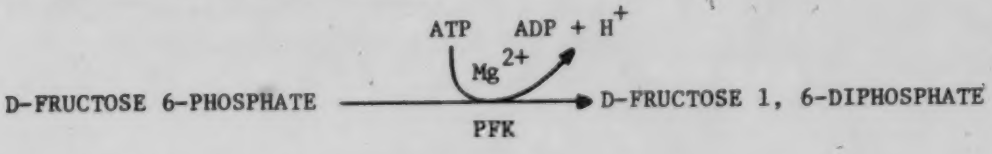
and SDH activates the oxidation of succinic acid to fumarate:

The nature of the aerobic training program (mild intensity, long duration exercise) causes adaptation of the muscle cell at the aerobic level as shown with laboratory animals as well as humans (Holloszy, 1967; Gollnick et al., 1973). Almost any type of aerobic training (swimming or running) sustained for many weeks (6 weeks to 5 months) causes a 50% to two-fold increase in the oxidative capacity of the muscle as shown by SDH and CS activities in the skeletal muscle involved in the exercise, depending on the intensity and duration (Holloszy, 1967; Howalski et al., 1969; Holloszy et al., 1970, 1971; Baldwin et al., 1972; Edgerton et al., 1972; Gollnick et al., 1973; and Piehl et al., 1974; Harri and Voltola, 1975; Terjung, 1976). The most commonly used aerobic training program is the one suggested by Holloszy (1967) as it produces training effects (increased work time to exhaustion and improved oxidative capacity of the muscle). In this program, the animals are trained on a treadmill at an intensity of 30 meters per minute at level or 8% incline for one or two hours per day, for 12 weeks. Ten to twelve bouts at 42 meters per minute, lasting 30 seconds, are interspersed through the workout. Heikkinen et al. (1975) reported increases of 18.3%, 6.3% and 7.4% of the D-ICDH, T-ICDH and MDH activities respectively in skeletal muscle of mice after 3 weeks of aerobic training.

Generally, only minor variations are made to this typical program and the speed of 30 meters per minute is a common factor as well as the daily duration of exercise. Some laboratories use swimming which is considered to be of mild intensity.

In humans, the bicycle ergometer is the most commonly used apparatus, the intensity of exercise varying between 50 to 75% of the maximal oxygen consumption. The subjects work for about one hour per day, four days per week (Gollnick et al., 1973) and the training program lasts up to five months.

Ozand and Narahara (1964) showed the rate limiting effect of phosphofructokinase (PFK) in glycolysis. It is not surprising, then, that phosphofructokinase became of interest to researchers in the biochemistry of exercise. PFK catalyzes the transfer of one phosphate from an ATP molecule to the D-fructose 6-phosphate to form D-fructose 1-6 diphosphate.



Its rate limiting effect makes PFK a key enzyme in the process of glycolysis. The effect of aerobic training, of the type described above, on the activity of PFK is not as clear as for SDH and citrate synthase. Results seem to be dependent on the type of muscle involved.

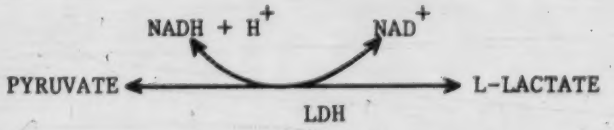
Baldwin et al. (1973), using a typical endurance training program, found a significant decrease in the red part of the quadriceps of rats while the soleus increased its PFK activity and the white part of the quadriceps stayed stable. Holloszy et al. (1971) obtained similar results for the gastrocnemius muscle of rats as Baldwin had found for the quadriceps. Harri and Voltola (1975) found a 13.6% decrease in PFK activity in gastrocnemius muscle of rats after 5 weeks

of swimming. The gastrocnemius muscle is mostly Fast Glycolytic ($\simeq$60%) in the adult rat (Ariano et al., 1973).

Gollnick et al. (1973) found a two-fold increase in PFK activity in the vastus lateralis of humans after five months of aerobic training (75% MVO_2) on a bicycle ergometer. This result is in contradiction to the preceding, and Gollnick et al. (1973) explained these findings as an increase in the glycolytic capacity of the slow twitch fibers. Despite the fact that the fiber typing in this study showed a higher percentage of Fast Glycolytic ($\simeq$60%) fibers, the authors conclude that this type of exercise especially mobilizes slow twitch fibers which would stimulate an adaptation of these fibers toward a higher degradation of glycogen for energy supply. This conclusion really means that the actual increase in the slow twitch is much larger than two-fold, as only 40% of the whole sample caused that increase. Contrary to these results, Gollnick et al. (1972) reported no difference between endurance trained athletes and untrained men in PFK activity of the deltoid and vastus lateralis muscles. Moesch and Howald (1975) found endurance athletes to have GAPDH activity 19% lower than sedentary men. Morgan et al. (1971) found no significant difference in PFK activity in the trained leg of humans compared to the untrained one after one month of training at 300 to 900 kg-m x min^{-1}.

Glycolysis can supply energy anaerobically only; its end product, pyruvate is then either transformed to lactate, or is oxidized and provides energy aerobically through the citric acid cycle. Muscles that are not highly oxidative rely on glycolysis

for energy with the production of lactate since pyruvate is oxidized inefficiently. These muscles are characterized by high lactate dehydrogenase activity, the enzyme which catalyzes the pyruvate – lactate reaction.



Lactate dehydrogenase (LDH) is, then, an important factor in studying the aerobic and anaerobic capacity of muscles. The effect of aerobic training on this enzyme seems to be very dependent on the type of muscle studied. LDH activity decreases significantly in high glycolytic muscles (FG and FOG), while it does not change in high oxidative, low glycolytic muscles (Baldwin et al., 1973; Holloszy et al., 1971; Edgerton et al., 1972; York et al., 1974). This was shown by the absence of change in the soleus and a significant decrease in the red and white parts of the quadriceps of the rat or in the soleus of Galago Senegalensis (Edgerton et al., 1972).

York et al. (1974) found that the decrease in the fast (oxidative glycolytic and glycolytic) muscle is due to a decrease in the isoenzyme of type Muscle (LDH-M) while the isoenzyme of type Heart (LDH-H) stays constant after aerobic training. Morgan et al. (1971) found no change in LDH activity after one month of training in human quadriceps muscle. Heikkinen et al. (1975) obtained the same results for the rectus femoris muscle of mice after 3 weeks of aerobic swimming. Karlsson et al. (1975) also found a higher percentage of LDH-H in a group of endurance trained athletes while the strength trained athletes showed higher levels of the M form.

In this study the higher the percentage of slow twitch (oxidative) fibers the higher was the percentage of LDH-H, thus showing better utilization of lactate and a lower capacity for fermentation. In muscle in which the proportion of slow twitch fibers was higher than 60%, no trace of LDH-M was found. However it is difficult to conclude that this is strictly due to training effects because of the cross-sectional nature of the study. Nevertheless, Peter et al. (1971) showed the predominance of LDH-M in the fast fibers (glycolytic and oxidative glycolytic) of guinea pigs while LDH-H was predominant in slow fibers, and concluded that LDH-H characterizes a high aerobic metabolism and LDH-M a high glycolytic metabolism.

Oxidation of free fatty acids is another source of energy supply for working muscle. Mole et al. (1971), after a typical endurance training program, showed a two-fold increase in the capacity to oxidize free fatty acids by a 100% increase in the activity of A-T-P dependent Palmityl-CoA Synthase, carnitine pamityl transferase, and palmityl-CoA dehydrogenase in the rat gastrocnemius and quadriceps muscles. Holloszy et al. (1971) reported the same results.

Unfortunately there is a scarcity of work on the enzyme complex of the β-oxidation pathway and its modification by training. Saville and Edington (1975) found a 41% increase in β-HOADH activity after 45 minutes of electrical stimulation of the rat gastrocnemius muscle of trained rats compared to untrained animals. Moesch and Howald (1975) reported 37% higher activity of the same enzyme in trained than in sedentary men.

Glucose can be an important source of energy for the working muscle. But before it enters glycolysis it has to be phosphorylated. Hexokinase catalyzes this phosphorylation to form glucose 6-phosphate. As shown by Bass et al. (1969) and others, hexokinase activity in muscle appears to be in constant proportion with the citric acid cycle enzyme activities and the respiratory chain (cytochrome c). It is not surprising then to observe increases in hexokinase activity following aerobic training, in order to maintain the relationship HK-citric acid cycle, as this physiological stress also stimulates citrate synthase and succinate dehydrogenase activities (Holloszy, 1967; Kowalski et al., 1969; Baldwin et al., 1972; Edgerton et al., 1972).

The "protection" of this constant proportion seems reasonable when one considers the sparing effect of aerobic training on glycogen stores in the muscle (Baldwin et al., 1975). The results obtained by Piehl et al. (1974) could be interpreted that way as an increased hexokinase activity (18%) accompanied by a 35% increase of glycogen synthase activity at rest in the trained leg of humans over the contralateral non-trained leg. The glycogen content of the trained leg was found to be 32% higher than the untrained leg at rest. They concluded that hexokinase would favorize replenishment of glycogen by increasing the glucose 6-phosphate pool in the muscle.

However hexokinase seems extremely sensitive to the stress of exercise as it increases in activity with training periods as short as nine days (Barnard and Peter, 1969) and increases in fast (red and white) as well as in slow muscles (Baldwin et al., 1973;

Peter et al., 1968; Lamb, et al., 1969; Barnard and Peter, 1969; Holloszy et al., 1971; Heikkinen et al., 1975; Morgan et al., 1971; Harri et al., 1975; Huston et al., 1975). Peter et al. (1968) obtained slightly higher increases in the white qudriceps than in the red quadriceps of guinea pigs after 21 days of training. This is not the case in Baldwin's study, where the red quadriceps of rats almost ripled its hexokinase activity while the white part of the muscle increased by 20% and the soleus by about 65% after twelve weeks of training. Barnard and Peter (1969) also found that the increased hexokinase activity stabilised after nine days of training and decreased when the training time per day was doubled between day 15 and 18 to stabilize again between day 18 to 24. Using three different work loads over a period of four weeks, Baldwin et al. (1973) observed the highest activity in the running program of 30 minutes every other day, followed by a 5 minute per day program, and finally by a 15 minute per day program (all higher than sedentary animals). Comparing these results to the time to exhaustion for each group they observed a lack of relationship between the level of training and hexokinase activity.

It then appears that hexokinase activity increases differently depending on the muscle type, the intensity and length of training (from 20% to 200%) and is not related to the training level.

Anaerobic Training and Enzyme Activity in Skeletal Muscle

Compared to aerobic training, anaerobic training is not very well documented in terms of its physiological and biochemical effects

on the skeletal muscle as well as on the whole organism. This type of training is characterized by high intensity exercise that can be sustained for short lengths of time. The intensity is close to or over the maximal oxygen consumption of the subject (90 to 150% MVO_2). At that intensity, the muscle depends on anaerobic metabolism. If the duration is very short the phosphagens (ATP, CP) (Karlsson, 1971) constitute the main source of energy; otherwise anaerobic glycolysis is used and lactate levels rise sharply (Saltin and Essen, 1971). Decrease of the pH and depletion of the phosphate energy rich pool causes the muscle to fatigue rapidly and to stop working.

The difficulty in inducing laboratory animals to exercise anaerobically is probably the principal reason for the lack of investigation in this area. Lately, however, a number of researchers have succeeded in training rats anaerobically (Saubert et al., 1973; Staudte et al., 1973; Wilkinson et al., 1975; Reardon, 1975) and this area of research is now expanding rapidly. The anaerobic characteristics of isometric training, due to limitation of blood flow by sustained muscle contraction, makes it relevant to our discussion. The studies on isometric training will be considered along with those on isotonic anaerobic training.

Enzymes involved in aerobic metabolism have been shown to increase their activity (optimal, in vitro) following anaerobic training. Staudte et al. (1973) found significant increases in the activity of citrate synthase in the rectus femoris as well as in the soleus muscle. This training program consisted of treadmill running at 80 meters per minute (at the end of the program) against

a 30^{o} incline. The rats were trained daily, twice in the morning and twice in the afternoon for 45 seconds with a rest period of 60 minutes. The program lasted 21 days.

The increase in aerobic capacity (CS) of both fast and slow twitch muscles, found in that study is understandable as they are both recruited during high intensity, short duration exercise as demonstrated in glycogen depletion studies (Gollnick et al., 1974).

However the lack of increase in glycolytic enzymes (in the slow muscle) could be attributed to the short duration of the actual anaerobic program; work done in our laboratory (Wilkinson et al., 1975 and Reardon, 1975) showed that a period of about 15 days is necessary to induce the animals to train at such a high intensity (60 - 80 m/min.). The actual anaerobic training appears then to be quite short (one week) in Staudte's study.

Wilkinson et al. (1975) also showed an increase in aerobic capacity of Fast Glycolytic fibers following ten weeks of anaerobic training. This increase in aerobic capacity of the trained rats compared to sedentary rats of the same age (15 weeks old) was in fact a maintenance of the percentage of Fast Oxidative Glycolytic fibers that the sedentary rat as well as the guinea pig (Maxwell et al., 1973) usually loses through growth. This change however cannot be quantified as the study consisted of histochemical measurements. Aerobic capacity was evaluated by the intensity of the staining of tissue for NADH-diaphorase activity (Wilkinson et al., 1975). The training regimen consisted of running at 80 to 100 meters per minute against a 30% grade for ten bouts of 15 seconds work/20

seconds rest, in the morning and in the afternoon (20 bouts/day).

Exner et al. (1973a, 1973b) found a significant increase in the activity of citrate synthase (approximately 30%) in the rectus femoris of male rats after an isometric training program, but no change in females. The higher aerobic capacity of female muscle at the beginning of the study seemed to play a role in the different effect of the program on both sexes. The absence of increase of the succinate dehydrogenase activity is not explained by the authors. The training program consisted of one in which the animals supported themselves against a 60^{o} incline with a weight of 90 to 200 grams attached to their tails. In each session the animals exercised until exhaustion. Three sessions not exceeding 5 minutes were repeated twice daily (12 hours apart).

Saubert et al. (1973) showed no increase in aerobic capacity of the red gastrocnemius, the white gastrocnemius, the red vastus and the soleus muscle of the rat after anaerobic training for 11 weeks. The training was run progressively from 61 meters per minute to 80.5 meters per minute (11th week) for 5 to 18 bouts (30 sec. exercise, 30 sec. rest). Changes in aerobic capacity were determined by assay of succinate dehydrogenase. This absence of change (increase) in SDH activity is in agreement with the previous studies reported as SDH activity does not appear to increase following anaerobic training.

Phosphofructokinase does not increase its optimal activity after anaerobic training as would be expected from such a stress. However, studies on this particular enzyme relative to anaerobic

training are few (Saubert et al., 1973). Nevertheless the soleus muscle seems to increase part of its glycolytic capacity as shown by significant increases in phosphorylase activity (Staudte et al., 1973; Saubert et al., 1973; and Exner et al., 1973) and in triose-phosphate dehydrogenase activity (Staudte et al., 1973).

Enzymes involved in the β-oxidation of the free fatty acids do not appear to undergo an increase in activity after anaerobic training as shown by Staudte et al. (1973) and Exner et al. (1973) for 3-hydroxyacyl-CoA dehydrogenase.

The effect of anaerobic training on fermentation of pyruvate to lactate is undecided since Staudte et al. (1973) showed no change in lactate dehydrogenase of both fast and slow muscles (rectus femoris and soleus) which is in contrast to the findings of Exner et al. (1973). Exner found a significant increase in LDH activity in the rectus femoris muscle of the rat.

In anaerobic exercise the muscle relies primarily on glycolysis (if sustained more than 20 seconds) through which glycogen as well as glucose can be catabolized (Reardon, 1975). Hexokinase activity would then appear to be an important factor in making glucose 6-phosphate available for glycolysis as well as for regeneration of glycogen.

Despite the scarcity of work in the area of anaerobic training, two studies report the effect of this stress on hexokinase activity in the muscle of rats (Staudte et al., 1973 and Exner et al., 1973). Unfortunately no discussion can be brought to bear on the results of these studies since they involved different anaerobic

training programs giving different results. Staudte et al. (1973) found a 50% increase in hexokinase activity of the rectus femoris as well as of the soleus muscle of the rat after 21 days of training. Exner et al. (1973a) found no differences in female rats trained isometrically for 25 days.

For reasons mentioned earlier, the results of Staudte's study are difficult to interpret in terms of the effects due to anaerobic training as the program is considered extremely short.

It is difficult to establish any conclusions regarding the effect of anaerobic training on activity of enzymes (HK, LDH, HOADH, PFK, SDH) of the energy supplying metabolism as only one anaerobic training program (Saubert et al., 1973) was of sufficient length to cause possible changes. It is also difficult to compare Saubert's study with the effects of isometric training, observed by Exner et al. (1973), since contractile events in isometric training are different compared to isotonic work. However it appears that fast glycolytic muscles benefit the most from this type of training by increasing their aerobic capacity.

CHAPTER III

METHODOLOGY

1. Animal Care

Fifty-eight male Wistar rats[1] (7 to 9 weeks old) were randomly assigned to three groups: I Sedentary control, II Exercised, III Trained. The animals were housed in individual cases and fed (ad libitum) a regular diet of laboratory chow (see appendix E for content) and water. The chow was replenished regularly so that the chow holders were never empty. The water was changed regularly (every 2 or 3 days) depending on the needs of each animal. Every animal was weighed on arrival and once a week thereafter (on Thursday morning, before training). All animals were given one week of adaptation to the new environment and to the reversed day-night cycle. The day-night cycle was of 12 hours light - 12 hours darkness (dark 6:00 a.m. to 6:00 p.m.). All animals were handled daily from day one until death.

When an animal was observed to be sick or seriously injured, it was isolated and sacrificed. This animal was anesthetized with ether and the body destroyed. Non-runners and bad runners were eliminated by the same procedures. After each training session every animal was individually checked for injuries, properly treated and well dried with a paper towel before returning it to its cage.

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[1] From Woodlyn Farms Ltd., R.R. 3, Guelph, Ontario.

2. The Training Programme

At the end of the adaptation week, the animals from groups II and III were exercised on a motor-driven treadmill (Quinton company) consisting of a wide endless belt on rollers divided into 10 compartments (75 x 10 cm). Motivation was provided by electrical stimulation by means of a shock grid located at the rear of each compartment. The voltage was about 50 volts (± 5 volts). All animals (groups II and III) were submitted to the same training intensity and schedule for the first two weeks (15 days exactly). These two weeks were an orientation period to train the animals to perform adequate sprint running at a very high intensity. The animals were taken progressively from 20 meters per minute at 30% incline to 80 meters per minute at the same angle. The training was initially aerobic and lasted from three to six minutes, whereafter the training became highly anaerobic and of short duration. The progression used may be found in appendix F.

The training schedule consisted of two training sessions per day, 6 to 8 hours apart. The morning session was held between 6:30 hrs. and 8:30 hrs. and the afternoon session between 3 and 5 o'clock. The animals were trained four days per week (Monday, Tuesday, Thursday and Friday). However, if an animal was observed to have a minor injury or showed signs of fatigue it was left in its cage for the following training session. If it did not recover within two days (48 hours) the animal was excluded from the study and sacrificed as mentioned earlier.

After the 15 days of orientation all animals could perform a minimum of ten consecutive work/rest intervals (15 sec/20 sec) at about 80 meters per minute (± 5 meters/min). The treadmill was automatically started and stopped by means of interval timers and the repetitions recorded on an electric counter so as to free the trainer to take appropriate care of the animals. At the end of the orientation period, half of the exercised animals (group II) were sacrificed. The other half were then put on a special programme to maintain performance at 80 m/min without any further training effect. This programme consisted of one series of 5 to 8 bouts (15 sec. on, 20 sec. off) per week, each Thursday morning.

The trained rats (group III) were continually trained thereafter for four more weeks at 80 to 90 m/min at a 30% grade. The regimen included 20 bouts per day (10 in the morning, 10 in the afternoon) with a schedule of 15 seconds on, 20 seconds off. This regimen was followed four days per week (Monday, Tuesday, Thursday and Friday).

3. Experimental Design

The experimental design is summarized in figure 1. Group I (sedentary) was divided into three subgroups of 8, 8 and 9 animals respectively. These control rats were sacrificed at the end of the adaptation week (8 animals), two weeks and six weeks after the adaptation week (8 and 9 animals respectively) to allow for consideration of the growth factor.

Figure 1 Experimental design

Groups	Number of Animals Sacrified at Different Times			
Time after arrival (approx.)	7 days	3 weeks	7 weeks	Total
Sedentary control (C_1 C_2 C_3)	8	8	12*	28
Exercised (E_1 E_2)	0	8	9	17
Trained (T)	0	0	7	7
TOTAL				52

*3 animals (C_{3A}) from the second group are included.

Group II (Exercised) was divided into two subgroups of 8 and 9 animals respectively. The first 8 animals were sacrificed after the 2-week orientation period. The rest of the group was kept until the end of the training period (4 weeks). The purpose of the first subgroup (E_1) was to analyze changes due to the orientation period in which the training bouts were partly aerobic. The second subgroup (E_2, from now on will be called: Exercised Control) was used to compare the performance of anaerobically trained rats to non-trained animals of the same age and capable of sprint running.

The trained group (T) (7 animals) was anaerobically trained for 4 weeks after the 2 weeks of orientation. Most of these animals could run at about 100 meters per minute for 10 intervals (15 seconds on - 20 seconds off) at the end of the 4 weeks of training.

The groups were formed at random (numbers mentioned previously represent the number of animals per group at the end of the study) from the whole group of animals, the second day after their arrival. As they arrived each animal received a number corresponding to the order in which they were transfered from the transportation box to a cage. Two days later a random order of these numbers was obtained from the computer. This random number list determined which animals would be in each group. Thereafter each animal was identified by its own number (holes punched in the ears).

Group irregularities: discovery of an error in the training intensity, after two weeks of the anaerobic training, forced the investigator to reject all exercised and trained animals. This was because overtraining effects were suspected after comparison of muscle weights between trained and control rats.

A new group of animals was ordered immediately to be paired (on body weight) with the control rats. Unfortunately, the animals delivered did not match the controls on weight. Because cost and time factors did not allow for ordering of new animals nor for waiting for the weights to be reached (the control rats had not been exposed to the laboratory environment for as long), a random sample of three animals was used as a control to compare with actual control rats (group C_3). These three animals were sacrificed at the same

time as the trained animals. This group was identified by the symbol C_{3A}.

4. Anaerobic Performance Test

Two days before sacrifice, trained animals as well as exercised control animals (E_2) were submitted to a performance test. The test consisted of running at 80 meters per minute on a treadmill at 30% incline, for intervals of 15 seconds interspersed by periods of rest lasting 20 seconds, until exhaustion. The total running time (sum of 15 seconds) determined the performance level of the animal. Each animal was individually run for the test. The animal was considered exhausted when it rolled on its back or if it refused to run for a period of more than 10 seconds despite the shock from the grid at the back of the treadmill.

5. Environmental Conditions

Temperature and relative humidity in the laboratory where the animals were kept and trained was verified regularly. The temperature in the room (20°C) never varied by more than one degree except on two occasions. At the end of the experiment the air circulation system of the whole building (on two different occasions) did not work for a period of 20 to 24 hours, during which times the temperature rose to about 25°C. The relative humidity, however, varied from 35 to 37.5% and never exceeded those values even during the times when the circulation system was not

functioning. The barometric pressure averaged 708.5 mm Hg.

6. Tissue Sampling and Sacrificing Procedure

The animals were sacrificed by decapitation (Guillotine), rapidly exsanguinated and the skin of the left hind leg removed quickly. The gastrocnemius muscle was then exposed and excised. Freed from connective and adipose tissue, the muscle was separated into two pieces (cross-section). The samples were weighed immediately and frozen in isopentane, cooled in liquid nitrogen and stored in a deep freezer (-80 to -90°C) until subsequent enzyme assay. The right gastrocnemius muscle was removed immediately following excision of the left one, and prepared as the left one but separated into three pieces, one being destined for fiber type determination. The average time for the decapitation of the animal, the excision, weighing and freezing of both legs was 12 minutes.

7. Procedures for Enzyme Assays

7.1 The "homogenate". The tissue sample to be assayed was ground into a very fine powder in a mortar containing liquid nitrogen. The ground tissue was then put on the surface of pre-frozen, pre-measured phosphate buffer (0.2 M, pH 7.0) and allowed to thaw in an ice-ethanol bath. The solution was then mixed and centrifuged at 1000 x g for 10 minutes (0°C). The supernatant was then poured in a test tube and stored in an ice-water bath in the refrigerator. A second volume of buffer was added to the pellets

and both were mixed thoroughly, then centrifuged for 10 minutes at 1000 x g (0^{o}C). The same operation was repeated a third time with the supernatants being added to each other each time.

The total dilution was 1:15 (g:ml) plus a few milliliters used to rinse the tubes. The first volume of buffer added was about 0.5 of the total dilution volume, the second about 0.3 and the third about 0.2. The homogenate or tissue-buffer solution was kept in the refrigerator in an ice-water bath until subsequent enzyme assay. All enzymes were assayed on the same homogenate preparation.

The 52 samples were prepared over a period of 24 hours. Because of uncontrollable circumstances, β-HOADH and Hexokinase were assayed within 3 days after homogenization, although β-HOADH was found[1] to lose considerable activity after 2 days of storage of the homogenate as mentioned previously. T-ICDH and LDH were assayed subsequently, on days 4 and 5. For homogenization as well as for enzyme assays, the samples were pooled so as to randomize the time factor effect. Each group of six samples contained one from each group (C_1, C_2, C_3, E_1, E_2 and T and C_{3A} when it was the case).

Pilot studies showed no significant change in LDH activity after 10 days of storage of the homogenate in the conditions mentioned here. All the other enzymes were fairly stable within 5 days except β-HOADH.

7.2 The Enzyme Assays. All enzymatic assays were done under the same conditions -- to allow direct comparison of the different enzymes under one given physical condition and to facilitate the use of ratios of enzyme activities. The physical conditions of pH and temperature were maintained at 7.0 and 37^{o}C respectively,

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[1] Unpublished results from this laboratory.

instead of optimal pH's and temperatures as usually done in enzyme assays. These specific physical conditions were chosen to approximate as well as possible the physiological conditions met at rest in the muscle tissue. It is usually said that physiological pH is around 7.4 (7.2 to 7.4). However this measurement is made in the blood. Knowing the tremendous buffer capacity of the blood, the value of 6.93 reported by Hermansen et al (1972) seemed very realistic. Thus a pH of 7.0 and a temperature of 37°C could be considered as normal (resting) conditions under which the muscle enzymes are functioning. It is important to note here that despite the fact that exercise physiologists (as well as physiologists in general) are reluctant to assay enzymes at a pH and temperature different from the optimal values reported by the biochemists for pure enzymes, there is no major problem in doing it. In fact pilot studies performed in our laboratory have shown that in many enzymes of the energy supplying metabolism the conditions maintained in the present study gave equal or higher activities compared to optimal conditions.

In the present study, the temperature was kept constant during the assays by means of a circulating bath (Polytemp by Polyscience[1]) directly hooked to the constant temperature cell holder of the spectrophotometer (UNICAM SP 800). The assay mixtures were modified from the original techniques[2] as detailed in appendix B so

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[1]Polyscience Corp., Niles, Ill. 60648, U.S.A.

[2]References of original techniques given in Table 1.

as to give optimal results under the conditions mentioned above. In all assays the mixture was incubated for 15 minutes at about 37°C (± 1°C) before the reaction was started and the cell placed in the cell holder which was kept at 37°C (in the spectrophotometer). All cells were mixed by inversion and the recording of enzyme activity began within 15 seconds of mixing. Recording was done over 1.5 to 5 minutes depending on the speed of each reaction. Assays of one enzyme on all the samples never exceeded 12 hours in duration.

Table 1

ASSAY METHODS

ASSAY	METHOD
Hexokinase	Joshi and Jagannathan, 1966
β-HOADH	Lynen and Wieland, 1955
LDH	Bernstein and Everse, 1975
NADP-ICDH	Lowry and Passoneau, 1972

All chemicals were freshly prepared[1] and kept in an ice-water bath ($T^{\circ} < 5^{\circ}C$). When NADH was involved no more than 15 to 20 assays were done with the same preparation because of the formation of

[1] Only freshly deionized distilled water was used in all preparations.

inhibitors (Fawcett et al., 1961). When assays were lengthy, fresh NADH was prepared every 2 to 3 hours.

In cases where background activities were observed (β-HOADH, HK) the reference cell contained exactly the equivalent of the sample cell except the specific substrate so that non-specific reactions were automatically subtracted from the total enzymatic activity of the solution (homogenate).

8. Histochemical Techniques

The fiber type distribution of the entire gastrocnemius muscle of seven animals was determined using standard procedures. This small sample was chosen because of the time needed to evaluate all the 52 animals and because this parameter was included only to estimate the type of muscle assayed. Three and four animals were chosen (randomly) in the group C_3 and T respectively. The staining was done on serial sections of 10 microns, prepared in a cryostat at a temperature of $-25^{o}C$. Myosin ATPase stain was done as suggested by Guth and Samaha (1969), α- glycerophosphate dehydrogenase as suggested by Wattenberg and Leong (1960), and NADH diaphorase by the method of Dubowitz and Brooke (1973).

9. Data Analysis

9.1 Calculation of enzyme activity. All enzyme activities

were reported in micromoles of substrate formed per minute per gramme of tissue (wet weight); $\mu mole \times min^{-1} \times g^{-1}$ w.w. The activity was calculated from the following formula:

$$\text{ACTIVITY} = \frac{\Delta 0.D \times V}{6.22 \times v} \div g/ml$$

where: $\Delta 0.D$ = change in optical density per minute at 340 nm.

6.22 = extinction coefficient of NADH at 340 nm.

V = total volume in the cuvette.

v = volume of homogenate added to the crevette.

g/ml = grammes of tissue per ml of homogenate solution as measured after centrifugation.

g = weight of wet tissue immediately after excision.

9.2 Enzyme Ratios. The following enzyme activity ratios were calculated: LDH/T-ICDH, HK/T-ICDH, β-HOADH/T-ICDH and LDH/HK The ratios were calculated for each animal and statistical analysis performed on these values. It was decided not to multiply some activities by a constant to give bigger numbers as often done by Pette (1971) and Bass et al. (1969) except for some ratios where the ratio itself was multiplied to avoid too many decimal points in crowded tables.

9.3 Statistical Analysis. A one way analysis of variance (ANOVA) (Winer, 1971) was run on the data of single enzyme activities as well as the ratios of enzyme activities. The Newman-Keuls test (Winer, 1971) was used as an a posteriori test to locate the significant differences between groups (DERS program documentation: ANOV15). When the χ^2 test revealed non-homogeneity of variance a

more conservative a posteriori test was used, ie. the Scheffé-test (Winer, 1971).

The Student's t test was used to check significant differences between trained and untrained animals (T and E_2) groups respectively for the run times (performance test) and the fiber type distribution.

The Pearson correlation coefficients (Winer, 1971) were calculated between enzyme activities and performance time. In all statistical analyses, a probability of 0.05 or smaller was required for significance.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS

The results of this study are mainly presented in the form of tables containing the mean and standard errors of the mean (S.E.$_{\bar{X}}$) for each group. Detailed results can be found in appendices C and D. Appendix C contains all raw data for each variable considered in this study. Appendix D consists of the summary of each analysis of variance as well as the results for the Newman Keul's test of the difference between ordered means.

1. Body and Muscle Weight

The data on body weight showed a lack of homogeneity of variance and therefore the Scheffé-test was used to find the significant differences among means. Body weight increased significantly ($p < 0.05$) with growth as shown by the differences between groups C_1, C_2 and C_3 (Table 2). Although the trained animals gained weight due to growth as shown by the difference between groups E_1 and T this difference was found not to be statistically significant at the 0.05 level[1]. Despite this increase

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[1] using the Scheffé-test.

the trained rats did not grow as large as the sedentary animals. For the group in which training was discontinued after two weeks, body weight showed a larger increase compared to the trained animals at the end of the experiment (Table 2 and Figure 2a). Although these differences were not found to be significant (Scheffé - $p > 0.05$), the group of exercised control animals were lighter than the sedentary control (C_{3A}) animals of the same age.

Despite the fact that the Scheffe-test showed no difference between the two sedentary groups C_3 and C_{3A}, comparison between the exercised and trained animals and the sedentary groups C_1, C_2 and C_3 could lead to erroneous conclusions for the reasons mentioned in the previous chapter. The attrition rate of this study was 10% as 52 animals out of 58 were considered for final analysis.

Muscle weight was used to decide if the second group of animals could be kept and compared with the first group, as mentioned in the previous chapter, on the basis that the muscle tissue itself was of interest in this study. The importance of this decision was shown by the lack of significant difference[1] between the group C_3 and C_{3A}. However this lack of difference could be due to the size of group C_{3A} (N=3) and it seems safer to compare the muscle weight of the trained animals with the animals of group C_{3A} to show the lack of overall hypertrophy due to the anaerobic training

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[1] Newman-Keul's test.

TABLE 2

BODY AND MUSCLE (GASTROC.) WEIGHT
OF SEDENTARY, EXERCISED AND TRAINED RATS

	GROUPS	BODY WEIGHT (grammes)	MUSCLE WEIGHT (milligrammes)
C_1	Sedentary Control 9 weeks[1] N = 8	$282^{o\blacktriangle*\square}$[2] (±6)	$1253^{o\blacktriangle*\square Z}$ (±30)
C_2	Sedentary Control 11 weeks N = 8	344^{o} (±5)	1734 (±54)
C_3	Sedentary Control 15 weeks N=9	402 (±15)	1862 (±80)
C_{3A}	Sedentary Control 13 weeks N=3	358 (±6)	1616 (±58)
E_1	Exercised 9 weeks N=8	$259^{o\blacktriangle*\square}$ (±6)	$1260^{*\blacktriangle o\square Z}$ (±65)
E_2	Exercised Control 13 weeks N=9	338^{o} (±8)	1598 (±65)
T	Trained 13 weeks N=7	307^{o} (±7)	1620 (±41)

($\pm$ S.E$_{\bar{X}}$)

o,▲,*,□,Z $P<0.05$: o with C_3, ▲ with C_{3A}, * with C_2, □ with E_2, Z with T.

[1] approximate age of animals.

[2] Scheffé-test $P<0.05$.

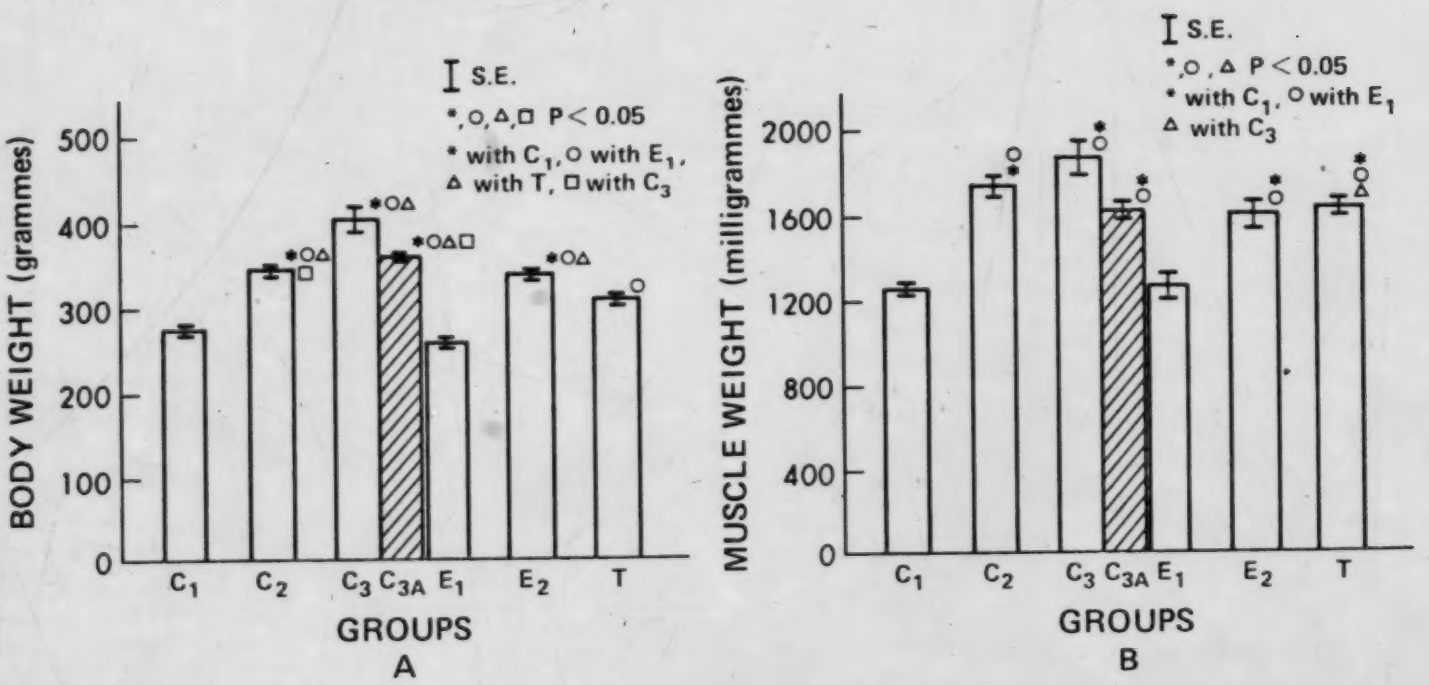

Figure 2 Body and muscle (gastrocnemius) weight of sedentary, exercised and trained rats

programme. The muscle weights for the different groups appear in Table 2 and Figure 2b.

2. Anaerobic Performance

The trained group performed anaerobically significantly longer compared to the exercised control animals as shown by mean run times of 199.3 seconds (±10.2) and 80.8 seconds (±6.6) for groups T and E_2 respectively. The anaerobic capacity, as expressed by the run time, of the trained rats was 147% higher than the exercised control animals (see Table 3 and Figure 3).

3. Enzyme Activities

The units used for enzyme activity are the international units, ie., micromoles of substrate transformed per minute, expressed per gramme of wet muscle (μ moles x min^{-1} x g^{-1} w.w.).

The analysis of the LDH activities for the different groups revealed a nonhomogeneity of variance, as shown by a significant chi square of 19.9 ($p < 0.01$). This seemed to be due to the larger variances of groups C_3 as well as E_2.[1] Despite a significant F value (overall difference) the Scheffé did not show any significant difference among means. The significant overall effect is

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[1] for enzyme activities as well as enzyme ratios, the group C_{3A} was not included.

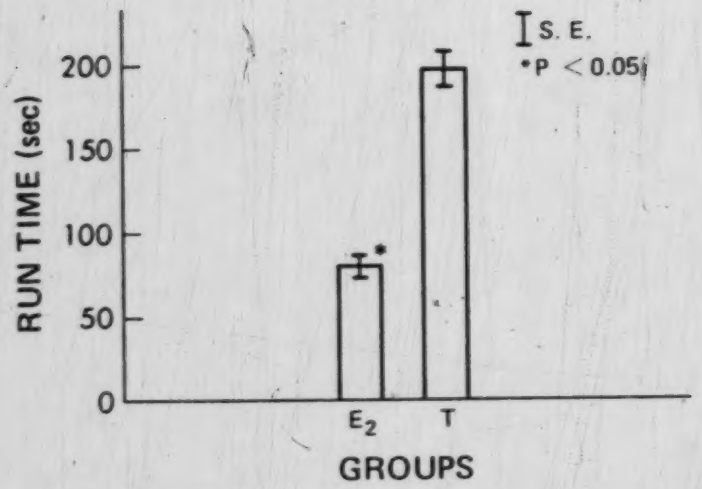

Figure 3 Run time at 80 m × min^{-1} at 30% grade; effect of anaerobic training

TABLE 3

ANAEROBIC PERFORMANCE OF
TRAINED VS. NON-TRAINED RATS

GROUP	RUN[1] TIME (Seconds)
Exercised Control E_2	80.8 (±6.6)9
Trained T	199.3 (±10.8)7

*$P < 0.01$ (t test)
(±S.E.)N

[1] at 80 m x min^{-1}, 30% grade.

attributed to the lack of homogeneity of variance. (See Table 4 and Figure 4a.)

The oxidative marker, NADP-specific Isocitrate dehydrogenase (T-ICDH) did not show any significant ($P > 0.05$) change among the different groups. The trained group (T) showed a 16% increase over the control group C_3. The exercised control animals (E_2) were found to have T-ICDH activity 15% lower than the trained animals (T) at the same age. (Figure 4b)

TABLE 4

ACTIVITY OF ENZYMES OF THE ENERGY SUPPLYING METABOLISM OF SEDENTARY, EXERCISED AND TRAINED RATS (GASTROCNEMIUS MUSCLE)
(μ moles x min^{-1} x g wet $weight^{-1}$)

GROUP	LDH[1]	T-ICDH	HK	β-HOADH
C_1 Sedentary Control 9 weeks N=8	1251 (±76.4)	14.8 (±1.7)	0.61 ▲■ (±0.05)	0.43 (±0.05)
C_2 Sedentary Control 11 weeks N=8	1251 (±46)	12.0 (±1.0)	0.46 *o▲■ (±0.04)	0.37 (±0.02)
C_3 Sedentary Control 15 weeks N=9	1458 (±148)	11.9 (±1.3)	0.42 *o▲■ (±0.04)	0.32 (±0.04)
E_1 Exercised 9 weeks N=8	1338 (±45)	14.1 (±1.7)	0.79 (±0.06)	0.42 (±0.04)
E_2 Exercised Control 13 weeks N=9	1699 (±119)	12.0 (±0.9)	0.63 ▲■ (±0.04)	0.34 (±0.02)
T Trained 13 weeks N=7	1398 (±53)	13.8 (±1.8)	0.82 (±0.06)	0.32 (0.03)

▲, o, *, ■ $P<0.05$ (Newman-Keuls) (± S.E.)

* with E_2, o with C_1, ■ with E_1
▲ with T

[1] not significant ($p>0.05$) Scheffé- test.

Glucose phosphorylation was affected by the training programme as shown by the differences in hexokinase (HK) activity among the different groups. The exercised animals (E_1) averaged 0.79 μ moles x min^{-1} x g^{-1} w.w. compared to 0.46 μ moles x min^{-1} x g^{-1} w.w. for the sedentary control animals (C_2) and an increase of 25% over the animals of group C_1. The exercised control animals (E_2) showed a

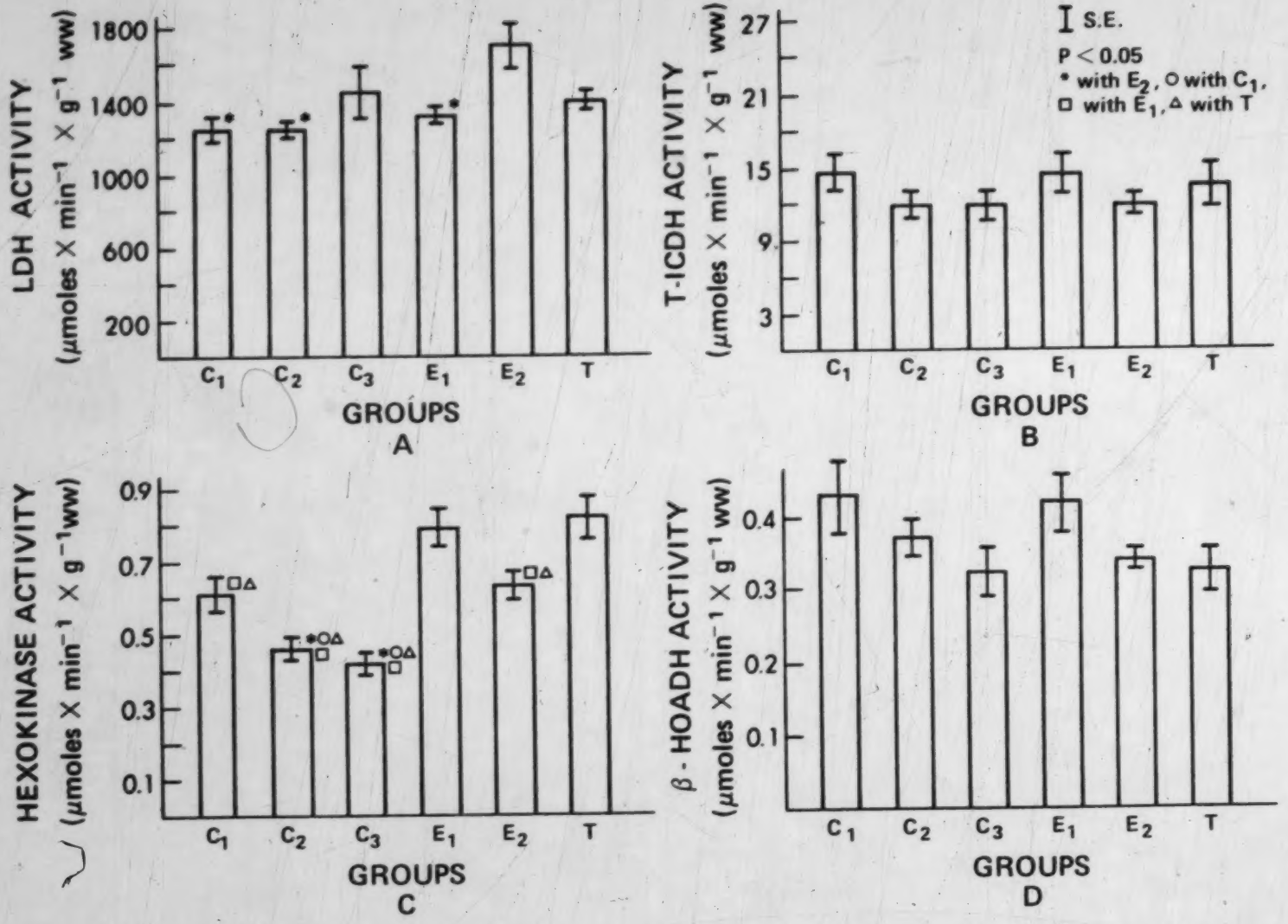

Figure 4 Activity of enzymes of the energy supplying metabolism of sedentary, exercised and trained rats (gastrocnemius muscle) (in μmoles × min^{-1} × g wet $weight^{-1}$)

decrease of 20% compared to group E_1, while the trained animals (T) had a significant increase of 4% over the animals of group E_1. A decrease in HK activity appeared with growth. That is, groups C_1, C_2 and C_3 showed activities of 0.61, 0.46 and 0.42 μ moles x min^{-1} x g^{-1} w.w. respectively, and these differences were significantly different ($p < 0.05$) only between C_1 and C_2 and C_1 and C_3. The exercised (E_1) as well as the trained animals (T) had significantly (p 0.05) higher hexokinase activity than the groups of sedentary control and exercised control (E_2) animals. The trained animals (T) showed an increase of 94% in hexokinase activity over the sedentary control animals of group C_3.

4. Enzyme Ratios

In Table 5, some ratios were multiplied by a constant to avoid too many decimal figures in the table. The ratio of LDH over T-ICDH did not show any significant group differences. However a decrease of 11% in this ratio was observed for the trained animals (T) compared to the exercised control animals (E_2).

Training had an effect on the HK/T-ICDH rates as shown by the results in Table 5 and Figure 5b. The trained group had significantly higher values for this ratio ($p < 0.05$) than the sedentary animals of the control groups C_2 and C_3. Although no other means were significantly different from each other, all exercised and trained animals had significantly

TABLE 5

RATIOS OF ENZYMES OF THE ENERGY SUPPLYING METABOLISM
OF SEDENTARY, EXERCISED AND TRAINED RATS
(GASTROCNEMIUS MUSCLE)

	GROUP	LDH/ T-ICDH	HK/ T-ICDH[1]	B-HOADH/ T-ICDH[2]	LDH/ HK[3]
C_1	Sedentary Control 9 weeks N=8	95.3 (±15.4)	4.5 (±0.5)	3.0 (±0.2)	214.6 (±23.8)
C_2	Sedentary Control 11 weeks N=8	109.2 (±9.2)	4.0* (±0.3)	3.2 (±0.2)	289.5 (±39.08)
C_3	Sedentary Control 15 weeks N=9	131.1 (±15.8)	4.1* (±0.6)	2.8 (±0.2)	376.3 (±58.7)
E_1	Exercised 9 weeks N=8	103.0 (±10.4)	5.9 (±0.6)	3.1 (±0.2)	174.7°[4] (±11.8)
E_2	Exercised Control 13 weeks N=9	153.4 (±21.1)	5.5 (±0.5)	2.9 (±0.3)	280.7 (±30.4)
T	Trained 13 weeks N=7	113.9 (±17.4)	6.6 (±1.0)	2.6 (±0.3)	176.9° (±16.6)

[1] x 10^2, [2] x 10, [3] x 10^{-1}, [4] Scheffé-test $p < 0.02$

* $p < 0.05$ with Trained Animals, ° with C_3

(± S.E.)

higher values than sedentary control animals with figures of 0.059 , 0.055 and 0.066 for E_1, E_2 and T respectively compared to 0.045 , 0.040 and 0.040 for C_1, C_2 and C_3 respectively. The trained animals (T) also showed an increase of 11% and 20% over the exercised (E_1) and exercised control (E_2) animals respectively.

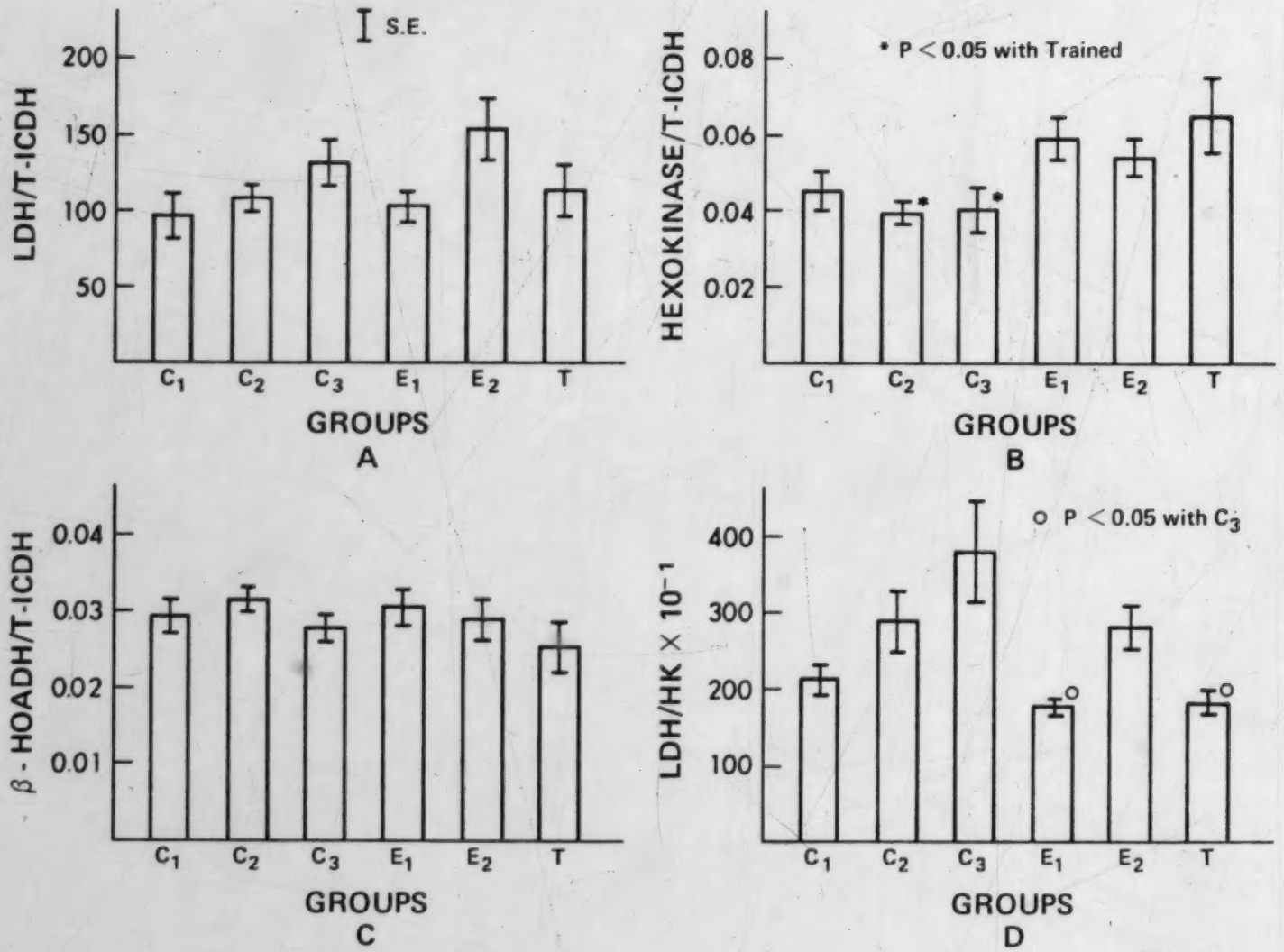

Figure 5 Ratios of enzymes of the energy supplying metabolism of sedentary, exercised and trained rats (gastrocnemius muscle)

The ratio β-HOADH/T-ICDH did not show any significant differences between groups. The group means ranged from 0.26 for the trained animals (T) to 0.32 for the sedentary animals of group C_2. Detailed results are presented in Table 5 and Figure 5c.

The LDH/HK ratio showed some significant differences despite the fact that the homogeneity of variance was not met here ($X^2 = 22.6$ $p<0.01$). The group of trained animals as well as the group of exercised rats (E_1) showed a significant[1] decrease in this ratio with the control group C_3. The trained animals (T), the exercised animals (E_1) and the sedentary control animals of group C_3 averaged respectively 176.9, 174.7 and 376.3.

5. Correlation of Enzyme Activities to Anaerobic Performance

The correlations of enzyme activities to anaerobic performance as expressed by the total run time at 80 meters x min^{-1} and 30% grade are given in Table 6. Only one enzyme activity was shown to be significantly ($p<0.05$) correlated with anaerobic performance, that being hexokinase with an r value of 0.48.

6. Muscle Fiber Distribution

The muscle fiber distribution of a total sample of seven animals showed a significant increase in the Fast Glycolytic fiber

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[1] Scheffé-test $p<0.05$.

TABLE 6

CORRELATION OF ENZYME ACTIVITIES
TO ANAEROBIC PERFORMANCE

ENZYMES	
LDH	-0.33
ICDH	0.19
HK	0.48*
β-HOADH	-0.12

*$P < 0.05$

portion (%FG) in the gastrocnemius muscle of the three anaerobically trained rats. The percentage of Fast Glycolytic fibers was 77.1 and 90.2 for the sedentary control animals (C_3) and the trained animals (T) respectively. This increase is reflected by a decrease in both Slow Oxidative (SO) and Fast Oxidative Glycolytic fibers (FOG) in this muscle.

TABLE 7

MUSCLE FIBER TYPE DISTRIBUTION OF THE GASTROCNEMIUS MUSCLE OF THE ANAEROBICALLY TRAINED RATS

GROUP	SO %	FOG %	FG %	TOTAL[1]
Sedentary (C_3) 4	3.8 ±0.6	19.2 ±2.5	77.1 ±3.1	5575 ±510
Trained (T) 3	0.8 ±0.7	8.9 ±1.8	90.2 ±1.1	3654 ±440

[1]TOTAL = Total number of fibers counted

$P < 0.05$

± S.E. , (group)N

DISCUSSION

1. Body and Muscle Weight

Figure 6 shows the steady increase in body weight in all groups as has been reported in similar studies (Exner et al., 1973a and b; Staudte et al., 1973; Houston and Green, 1975). However Exner et al. (1973a and b) reported a non-significant lower body weight for trained (isometric) animals while Staudte et al. (1973) found trained animals to have significantly lower body weights after 21 days of sprint training. Houston and Green (1975) reported similar findings for intermittent short-term exercise. The lack of significance found in this study is attributed in part to the a posteriori test (Scheffé) which is extremely conservative (Winer, 1971). Houston and Green (1975) reported differences in body weight after only 25 days. Differentiation appeared after two weeks of exercising in the present study (see Figure 6). The body weight of the control animals of group C_3 was plotted in Figure 6 to show the almost perfect parallelism in body weight with group C_{3A} as well as the exercise control group E_2. After cessation of training (group E_1), body weight patterns appeared to increase, in contrast to the trained group (T) in which body weight did not increase as rapidly.

The lack of overall hypertrophy observed in this study is in agreement with the findings of Staudte et al. (1973) for male rats and Exner et al. (1973a) for female rats. However Houston and Green (1975) found a slower increase ($p < 0.05$) of muscle weight (gastrocnemius) in trained animals from the age of 7 weeks to 12 weeks than

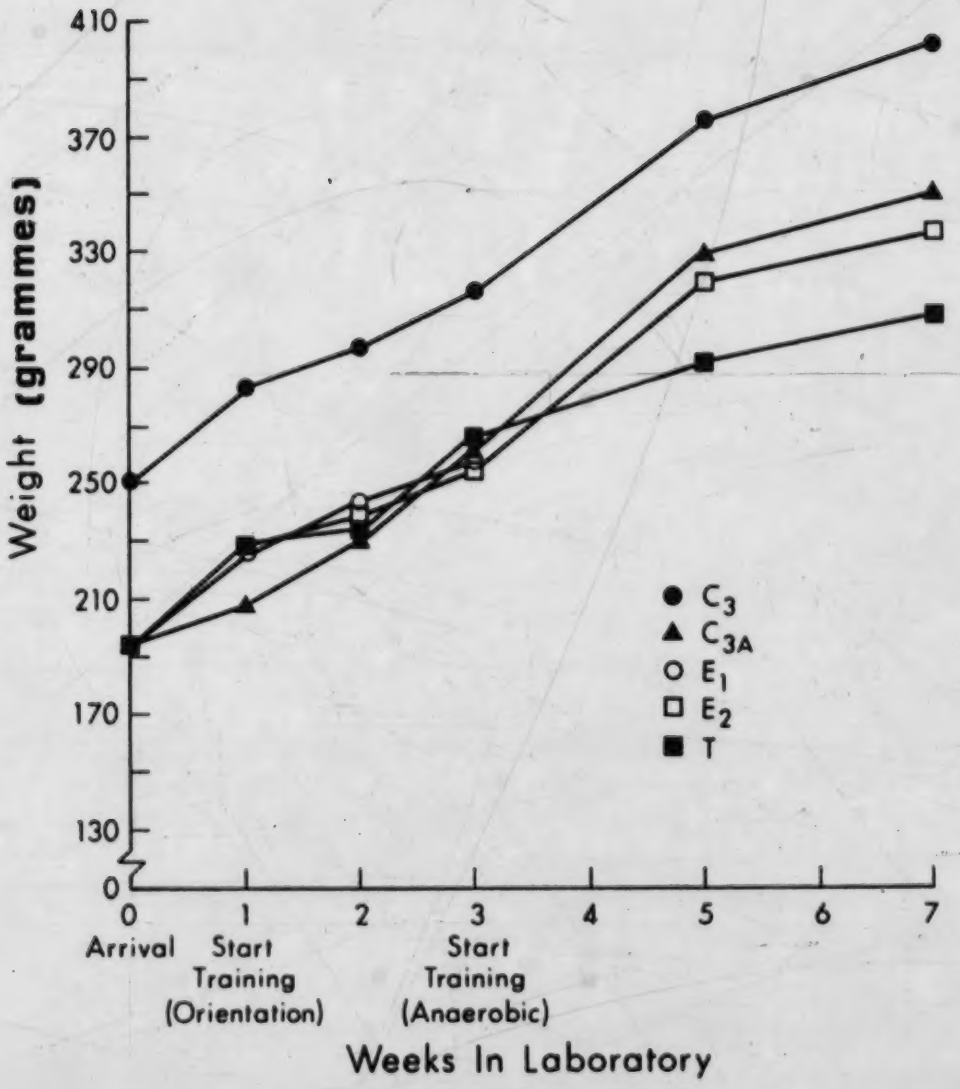

Figure 6 Interaction of growth and training on the body weight of rats

in control animals. Muscle weight increased constantly during growth.

2. Enzyme Activities; Growth Effect

No attempt will be made to compare the results of enzyme assays more extensively than was done in Table 8. A brief overview of this table shows the enormous discrepancy in enzyme activities even between studies in which fairly similar techniques were used. The physical condition of the assay (pH and T^{o}), the type of muscle, as well as the species, and the age of the animal are very important factors affecting enzyme activity in the tissue. However, the results of this study will be compared to others on the basis of the rate of change caused by the experimental condition.

The effect of growth on enzyme activity, as shown by the difference between groups C_1, C_2 and C_3 was significant only in the case of hexokinase. The decrease of 24% in HK activity took place between the ninth and the eleventh week. Thereafter hexokinase activity stabilized as there was no significant change between the groups C_2 and C_3. This decrease, as well as changes in the other enzymes (even though they were not statistically significant) show the same trends as measured by others. Bass et al. (1970) reported increased LDH activity along with a decrease in HK, β-HOADH and the oxidative enzymes in the fast muscle of chickens up to three weeks after hatching. Bernstein and Kipnis (1973) found a 30% decrease in HK II isozyme in rats during growth (150 to 450 grammes), but it was not specified which muscle was assayed. Dalrymple et al. (1974)

TABLE 8

COMPARATIVE TABLE OF ENZYME ACTIVITIES
OF SKELETAL MUSCLE IN SEDENTARY ANIMALS
FROM DIFFERENT STUDIES

ENZYME	SPECIES[1]	MUSCLE	FIBER TYPE %FOG	%FG	%SO	ACTIVITY[4] µmoles/min/g	pH	T° °C	AUTHOR
LDH	G.P.	white vastus	29	71	0	449±41(7)	7.4	37	Peter + al (1972)
	Man	quadriceps				236±41*(10)	not given		Morgan + al (1971)
	Rabbit	adductor magnus	not given			1365	7.6	25	Bass + al (1969)
	Rat	gastrocnemius	19.18	77.08	3.75	1458±148*(9)	7.0	37	Jobin
HK	G.P.	white vastus	29	71	0	0.297±.057(5)	7.4	37	Peter + al (1972)
	Man	vastus lateralis				1.61±0.57(24)	7.2	25	Moesch + al (1975)
	Man	quadriceps				0.750±0.19*(10)	not given		Morgan + al (1971)
	Rabbit	adductor magnus	not given			0.28	7.6	25	Bass + al (1969)
	Rat	gastrocnemius	19.18	77.08	3.75	0.42±.036*(9)	7.0	37	Jobin
T-ICDH	Rat	gastrocnemius	19.18	77.08	3.75	11.9±1.3*(9)			Jobin
β-HOADH	Rat	gastrocnemius	not given			8.8±0.8(8)	7.0	25	Staudte + al (1973)
	Rat	gastroc-plant.				2.107(4)	7.4	37	Saville + al (1975)
	Man	vastus lateralis	not given			21.3±10.2(24)	7.2	25	Moesch + al (1975)
	Rabbit	adductor magnus	not given			1.1	7.0	25	Bass + al (1969)
	Rat	gastrocnemius	19.15	77.08	3.75	0.32±.04(9)	7.0	37	Jobin

(1) G.P.: Guinea Pig, (N), * ± S.E.,
(2) After 2 weeks of aerobic training
(3) N.G. - Not given
(4) per g wet weight

reported an increase in LDH activity during growth in pigs. However, it was shown here that the growth effect was very similar in the case of Hexokinase and T-ICDH, where there was a more or less stabilization after 11 weeks. On the other hand, LDH activity changed (not statistically significant) only between the eleventh and fifteenth week while β-HOADH showed a progressive decrease (not significant) from week 9 to 15. The absence of significant differences in the case of LDH could be due to the use of the Scheffé test for this test is very conservative (Winer, 1971). The overall metabolic significance of these changes was not shown to be very important as there was no significant differences with growth in the enzyme ratios: LDH/T-ICDH, HK/T-ICDH, β-HOADH/T-ICDH and LDH/HK. However the trend indicated an increase in the relative importance of anaerobic glycolysis versus oxidative metabolism as shown by a 15% and 20% increase in the LDH/T-ICDH ratio between 9 weeks and 11 weeks and between 11 weeks and 15 weeks, respectively. This appears to be in agreement with Bass et al. (1970). The increase in the LDH/HK ratio of 35%[1] between 9 and 11 weeks and of 30% between 11 and 15 weeks is in harmony with a more pronounced capacity toward anaerobic glycolysis of the tissue. Although β-HOADH/T-ICDH showed a slight increase (7%) between 9 and 11 weeks, and a decrease between 11 and 15 weeks (12%), the two metabolic pathways (β-oxidation of FFA and the Krebs cycle) remained in harmony during growth in the gastrocnemius muscle of the rat. The interdependence of the two pathways toward

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[1] Scheffé test, $p \geq 0.05$.

energy release is a necessity since β-oxidation does not directly produce ATP.

3. Enzyme Activities; Training Effect

The effect of training alone is more difficult to evaluate compared to the effect of growth alone (the trained animals were all growing). This is because of the marked interaction between the natural process of growth and the physiological stress of anaerobic exercise. The comparison of enzyme activities between groups T and C_3 as well as E_1 and C_3 (to analyze the difference between sedentary, trained and exercised animals) was validated by the lack of a significant difference between groups C_3 and C_{3A}[1] (see Table 9 and Figure 7).

The difference in enzyme activities between the trained animals and the control group C_3 can be compared to results from other studies on high intensity short-term exercise training. The 94% higher HK activity in trained (T) over sedentary animals (C_3) measured in this study is much higher than what was found by Staudte et al. (1973). The authors reported a 47% increase in HK activity in the rectus femoris muscle of the rat. The higher increase in this study is explained by the fact that the intensity of the training programme was higher and was maintained for a longer period. The non-significant (statistically) higher activity (16%) in the T-ICDH activity in the gastrocnemius muscle of group T is, for some

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[1] C_{3A} was age matched with T and E_2 as mentioned earlier in METHODOLOGY.

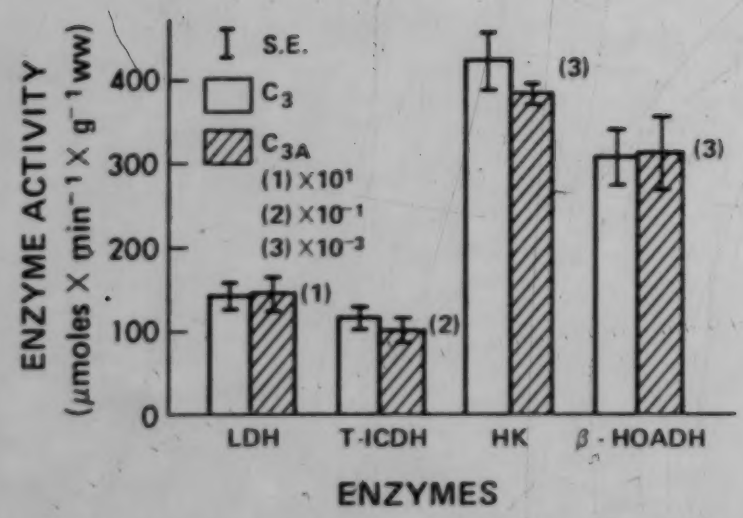

Figure 7 Enzyme activities of the gastrocnemius muscle of sedentary rats of groups C_3 and C_{3A}(≅ 2 weeks difference in age)

reason, intermediary to the 10% ($p>0.05$) and 24% ($p<0.05$) increases in SDH and CS activities found by Staudte et al. (1973) while Saubert et al. (1973) obtained a 9% increase ($p>0.05$) in SDH activity of the white gastrocnemius muscle of the rat. The higher activity of T-ICDH in the present study, although not statistically significant, is attributable to the four weeks of anaerobic training since no difference in this enzyme was observed in group E_1, compared to the sedentary control group (C_1) of the same age. Two weeks were probably not enough to stimulate an increase in this aerobic enzyme. Furthermore, the results could be affected by the fact that T-ICDH was shown to be less sensitive to aerobic training than D-ICDH (Dohm et al., 1973). But it is not known if this could hold for anaerobic training.

The absence of change in LDH as well as β-HOADH is in agreement with the findings of Staudte et al. (1973) for both enzymes and of Saubert et al. (1973) for LDH. The effect of anaerobic training on individual enzyme activities is thus shown to be very different from aerobic training since the citric acid cycle and the β-oxidation of fatty acids were not as markedly affected. The differences between aerobic training and the anaerobic training of this study can be stressed even better by comparing the direction of change in enzyme ratios. The findings of Moesch and Howald (1975) and Bass et al. (1976) are in opposition to the changes reported in the present study. These authors measured lower (statistically significant) HK/CS and HK/SDH ratios in endurance athletes compared to sedentary men, showing an increased importance of the

aerobic metabolism in trained men. The anaerobically trained rats (T), on the contrary, were found to have a higher HK/T-ICDH ratio than the sedentary animals of the same age (C_3), showing a lower capacity to handle glycolytic end products aerobically.

It is worth noting that the comparisons made above do not consider the growth factors in which none are made in most other studies. In the present study growth was shown to interact with training as mentioned earlier.

TABLE 9

ENZYME ACTIVITIES (μ moles x min^{-1} x g^{-1} w.w.) OF THE GASTROCNEMIUS MUSCLE OF SEDENTARY RATS OF ABOUT 2 WEEKS DIFFERENCE IN AGE

ENZYME	GROUPS C_3 (N=9)	C_{3A} (N=3)
LDH	1458 (±14 8)	1467 (±202)
T-ICDH	11.9 (±1.3)	10.4 (±1.6)
HK	0.42 (±0.04)	0.39 (±0.01)
β-HOADH	0.32 (±0.04)	0.33 (±0.04)

cf Figure 7

($p > 0.05$)

4. Enzyme Activities; Growth-Training Interaction

Effects of anaerobic training, then, cannot be evaluated only by comparison of the trained animals (T) with the sedentary control animals of comparable age (C_3). In fact the training effect appears to influence the rate of change of enzyme activities during growth rather than a real increase in metabolic capacity of the muscle. By comparing the trained animals (T) with the initial sedentary control rats (C_1), it is observed that only HK activity is higher in the trained group. For all other enzymes, the trained group (T) had either slightly higher (LDH) or lower (β-HOADH, T-ICDH) activities than the animals four weeks younger (C_1).

Thus, it is difficult to talk about an increase in enzyme activity with anaerobic training (except for HK). The effect of exercise (anaerobic) appears to be a slowing down of the "normal" (in sedentary animals) rate of decrease of T-ICDH activity and the "normal" rate of increase of LDH activities (see figure 4). However, training did not seem to affect β-HOADH activity since the same pattern was observed in sedentary control animals (C_1, C_2, C_3) and exercised and trained animals (E_1, E_2, T). Training (anaerobic) appears, then, to maintain some metabolic characteristics of the young animal. Furthermore, these effects appeared to be due to the four weeks of intensive anaerobic training in the case of LDH and T-ICDH. This is because of the absence of change between C_1 and E_1 and the return to more sedentary values in the case of E_2. Only hexokinase had gained significant activity (29%) at the end of the

two weeks of orientation compared to sedentary animals of the same age (C_1). In this case, it appears that the four weeks of anaerobic training just maintained this gain (see Figure 4c and Table 4).

The effect of anaerobic training on the metabolic pattern of the gastrocnemius muscle appears to be a maintainance of the characteristics of younger animals. Since none of the enzyme ratios were found significantly different in group T as compared to group C_1. However, some trends are worth considering. The trained animals (T) showed an increased hexokinase/T-ICDH ratio of 46% over the sedentary control animals (C_1). The trained rats (T) also decreased the LDH/HK ratio by 18% as compared to group C_1. In all cases group E_2 tends to go back to more sedentary values for animals of the same age (see Figure 5).

These effects of training over the growth changes on muscle metabolism are mainly due to the sharp increase in HK activity (61%) of the trained group (T) over the sedentary control group C_1. Because of the decrease in HK activity with growth, this increase is 94% when comparing group T to groups more approximately the same age at the time of sacrifice. Of significance is the 16% ($p > 0.05$) increase in T-ICDH for the trained animals as compared to the sedentary controls C_3. This difference no longer exists when T is compared to C_1 but a decrease is shown instead. This appears to be a very good example of the effect of training in which the result is an alteration of the rate of change of enzyme activities during growth.

The anaerobic training regimen used in the present study appears to cause a particular shift of the energy supplying metabolism. The increase ($p<0.05$) in the HK/T-ICDH ratio is interpreted as an uncoordinated adaptation between glucose phosphorylation and oxidative enzymes. The increase in HK activity (94% for T over C_3) was not proportionally geared toward an improvement in oxidative capacity (T-ICDH increased only 16%, T over C_3). In addition, the decreased LDH/HK ratio (T over C_3) indicated that the augmentation in glucose phosphorylation capacity was not accompanied by a proportional increase in the capacity of the tissue for fermentation of the end products of glycolysis.

5. Fiber Type Population of the Gastrocnemius Muscle of the Rat

The gastrocnemius muscle showed a high proportion of Fast Glycolytic fibers: 77% in the sedentary animals (14 to 15 weeks old). This percentage is greater than the findings of Ariano et al. (1973). These authors reported that the rat gastrocnemius muscle contained 58% FG, 37.5% FOG and 4.5% SO fibers. However there was no mention of the age of the animals (N=3). This makes comparisons difficult since the percentage of Fast Oxidative Glycolytic fibers was shown to decrease in favor of SO and FG fibers with growth in the plantaris muscle of rats (5 to 15 weeks old) (Wilkinson et al., 1975). The same phenomenon could also be expected in the gastrocnemius muscle.

The higher FG population in the trained animals (T) (90.2% FG, 8.9% FOG, 0.8% SO) is difficult to interpret since histochemical techniques are qualitative in nature. However, Prince et al. (1976) measured a higher percentage of FG fibers in weight lifters than in runners ($p < 0.001$) or sedentary men ($p < 0.05$). No glycolytic enzyme such as PFK or TPDH was included in the present study. Thus, no estimation of the actual glycolytic capacity of the muscle could be made.

6. Performance Time

The large increase in the performance time of the anaerobically trained animals is the most striking effect of this particular training regimen. Anaerobic capacity, or the capacity to exercise longer anaerobically, increased a great deal by anaerobic training. It is difficult to apply these results directly to the sprint athlete. However it appears that proper training would make such an athlete able to perform longer at optimal speed. The changes observed in the energy-supplying metabolism of the gastrocnemius muscle of the laboratory animals in this study give us an insight into the metabolic cause of this increased performance.

7. General Discussion

Because the assays were performed at physiological pH and temperature, it is assumed that the enzyme activities reflect the capacity of the muscle in "normal" resting conditions. Such a shift in the metabolic pattern, then, suggests a special meaning. One

would expect the different pathways to increase their capacity in a coordinated fashion if the energy requirements could be considered to be derived from the whole system. The ratios of enzyme activities give the direction of this shift. Pette (1971) suggested that changes in energy supplying metabolism "may be" understood as optimum adaptation of energy-supply to requirements of muscle function.

The importance of hexokinase to the energy supply of muscle after training is often underestimated. The conclusion of Baldwin et al. (1973) that hexokinase activity is not related to the level of training (time to exhaustion) helped to back up this idea. However, hexokinase appeared to be the key point for adaptive changes following anaerobic training in the present study. This was substantiated by the significant correlation between enzyme activity and the run time under anaerobic conditions ($r=0.48$, $p<0.05$). However, hexokinase is only one of the factors in the realization of optimal performance. Only one muscle was considered in this study, while most of the muscles involved in knee extension as well as ankle extension were probably also highly involved. The aerobic capacity of other muscle groups could play a significant role. The gastrocnemius was shown to be highly glycolytic. Factors such as the removal of lactic acid and its oxidation by other muscles cannot be ruled out.

Hexokinase plays a significant role in the energy supplying metabolism, and could be considered as a rate limiting enzyme when glucose is necessary to supply a sufficient level of G-6P. Newsholme and Start (1973) proposed this possibility. The same authors also made mention of the highly synchronized relationship of PFK and HK

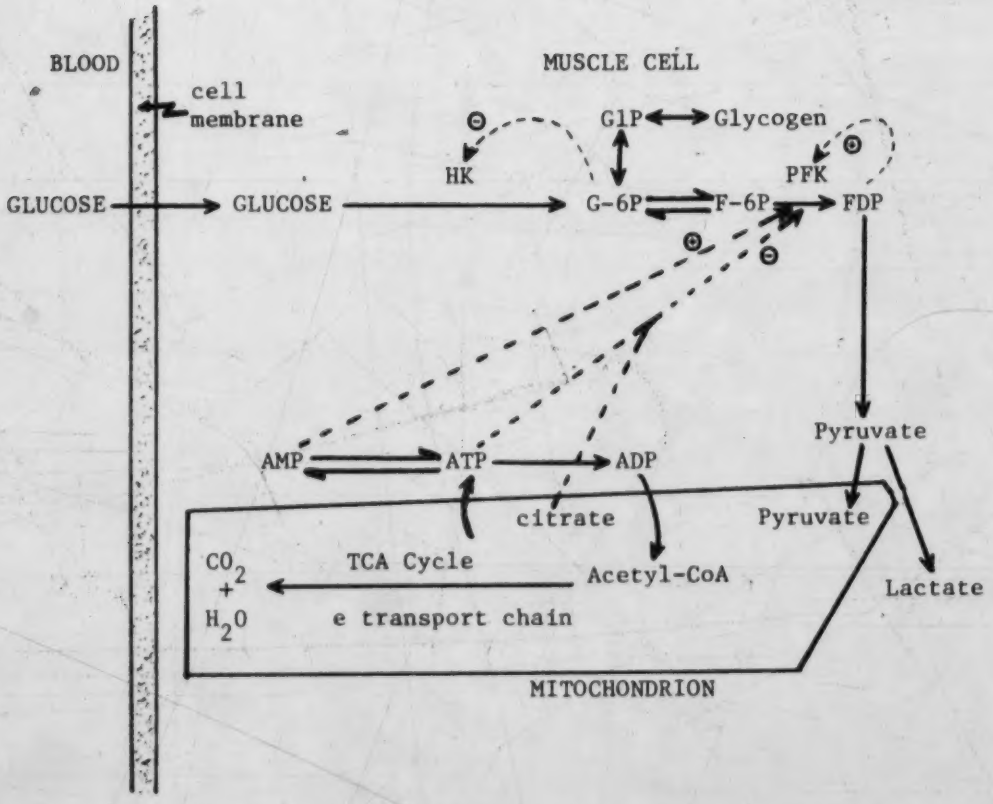

Figure 8. CONTROL OF GLYCOLYSIS IN MUSCLE AT THE HK-PFK REACTIONS. Adapted from Newsholme and Start, 1973.

> "HK-PFK concerted control system provides a flexible mechanism of control of glycolysis, permitting glycogen synthesis when glycolysis is inhibited, and enabling the specific hormonal and nervous control of phosphorylase to modify PFK activity through Glucose-6-Phosphate concentration." (Newsholme and Start, 1973)

in the control of glycolysis (see above diagram). However Newsholme and Start (1973) state that the low activity of hexokinase in the muscle (compared to other enzymes; PFK/HK = 37) means that anaerobic glycolysis from glucose would not supply sufficient energy for contraction.

The shift observed in the present study suggests that hexokinase would be important in the replenishment of the muscle glycogen stores during the hours of recovery between exercise sessions and training days. The changes in the ratios of LDH/HK as well as HK/T-ICDH support the idea that the hexokinase activity increase is involved somewhere else other than in the direct production of energy through glycolysis and oxidation of its end product-pyruvate. Piehl et al (1974) have demonstrated a relationship between the increase in hexokinase and glycogen synthase following aerobic training. This relationship may even be higher following anaerobic training.

Since glycogenolysis is the main source of energy in short duration, high intensity exercise, higher levels of muscle glycogen stores would be beneficial for performance under these conditions. The higher hexokinase activity measured in the resting muscle of the anaerobically trained animals would enhance that function.

The major adaptation to anaerobic training appears to be a higher capacity to replenish glycogen stores in the muscle and probably to increase these stores.

In the present study none of the variables measured showed any sign of abnormality, and furthermore all adaptations were positive and led to higher anaerobic work capacity. However, extrapolation to growing children is difficult and should be done with extreme caution. Parameters such as bone ossification, heart metabolism and functional capacity were not measured and are important factors in growing children. Nevertheless, the trained animals appeared to be healthy at the end of six weeks of high intensity training. Of more importance than the intensity per se

would perhaps be the progression toward it.

SUMMARY

Fifty-two male Wistar rats (180-260 gr) were divided into sedentary control, exercised, exercised control and trained groups. Animals were fed a regular chow diet ad libitum. Exercised and trained animals were progressively trained to run on a treadmill at 80 m/min (30% grade). This graded training period lasted 2 weeks. The animals were running 20 intervals of 15 sec. on -- 20 sec. off, 10 in the morning, 10 in the afternoon, 4 days a week (M. T. Tr. F.). The trained animals (T) continued training for 4 weeks at 80 m/min. (30% grade) while half of the exercised animals (E_1) were sacrificed after they reached the speed criterion (80 m/min). The other half (exercised control E_2) were exercised once a week for about 5 to 8 bouts at the same speed and angle as the trained animals (T) and were sacrificed at the same time as the trained animals (T). At the end of the training programme, trained (T) and exercised control (E_2) animals were given a performance test (run time at 80 m/min, 30% incline). The gastrocnemius muscle was excised 24 hours after a normal training session and kept frozen (-90°C) until assayed for HK, T-ICDH, LDH and β-HOADH activities. Assays were performed at physiological pH and temperature on the supernatant of whole homogenate. Training was shown to alter the rate of increase in body weight with age and the growth patterns in muscle metabolism. The trained animals (T) performed for 199.2 sec. compared to 80.8 sec. for the exercised control animals (E_2). The trained animals (T) showed a 94% higher HK

activity ($p < 0.05$) compared to the sedentary animals of approximately the same age (C_3) and 61.19% higher than sedentary animals 4 weeks younger (C_1). LDH/HK and HK/T-ICDH ratios were found to be, respectively, lower ($p < 0.05$) and higher ($p < 0.05$) in trained animals (T) over sedentary animals of approximately the same age (C_3). No overall muscle hypertrophy was found with training. It was shown that anaerobic training stimulated glucose phosphorylation. Performance (anaerobic) was found to be related to HK activity in the gastrocnemius muscle of the rat ($r=0.48$, $p < 0.05$).

CONCLUSIONS

Analyses of the results obtained in this study resulted in the following conclusions:

Anaerobic training for six weeks caused a slower weight gain of growing rats after two weeks. Cessation of training after two weeks did not affect the rate of weight increase for the following four weeks.

Anaerobic training during growth altered the change in enzyme activity and in the metabolic pattern of the rat skeletal muscle as compared to sedentary animals. Training (six weeks) caused an important shift in the energy supplying metabolism of skeletal muscle toward higher glucose phosphorylation.

Two weeks of graded exercise toward anaerobic training maintained the initial level of T-ICDH in the skeletal muscle during growth.

Anaerobic training increases the capacity to work at high intensity.

Hexokinase activity in the fast glycolytic muscle is related with work time at high intensity.

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APPENDIX A

LIST AND SOURCE OF CHEMICALS USED

LIST OF CHEMICALS USED

CHEMICAL	SOURCE[1]
N-ACETYL-S-ACETACETYL-CYSTEAMINE	SIGMA A-5011
ATP (Na_2 Salt) from equine muscle	SIGMA A-3127
DITHIOTHREITOL	SIGMA D-0632
FRUCTOSE 6-PHOSPHATE (K_2 Salt) (Grade VI)	SIGMA F-1502
PHOSPHOFRUCTOKINASE (Type III, Rabbit)	SIGMA F-6877
L-GLUCOSE	SIGMA G-5500
GLUCOSE-6-P-DEHYDROGENASE (Type XI)	SIGMA G-8878
α-GLYCEROPHOSPHATE DH + TRIOSE-P-ISOMERASE (Type X) (α-GDH-TPI)	SIGMA G-6755
β-HYDROXYACYL-CoA DEHYDROGENASE (Grade III)	SIGMA H-4626
ISOCITRATE (Na_3 Salt) (Type I)	SIGMA I-1252
ISOCITRATE DEHYDROGENASE (Type IV)	SIGMA I-2002
$MgCl_2 \cdot 6H_2O$	SIGMA M-0250
β-NADH (Na_2 Salt)	SIGMA N-8129
NADP (Na Salt)	SIGMA N-0505
$MnCl_2$	SIGMA 150-2
PYRUVATE (Type II) (Na Salt)	SIGMA P-2256

[1]SIGMA Chemical Company, St. Louis, Mo., U.S.A.

APPENDIX B

DETAILS OF BIOCHEMICAL ASSAYS

β-HYDROXYACYL-CoA DEHYDROGENASE (β-HOADH)
(E.C. 1.1.1. 35)
(L-3-hydroxyacyl-CoA: NAD oxidoreductase)

Modified from: Lynen F. and O. Wieland
β-Ketoreductase
in Methods in Enzymology
Ed. S.P. Colowick and N.O. Kaplan
Vol. 1, p. 566, 1955
and
Sigma Chemical Test

Principle: ACETOACTYL-CoA ⇄ (NADH+H$^+$ / NAD, B-HOADH) β-hydroxybutinyl-CoA

β-hydroxyacyl-CoA dehydrogenase catalyzes the reversible oxidoreduction between acetoacetyl-CoA or homologous β-ketoacyl-CoA derivatives and NADH.

The rate at which NADH disappears in this reaction is followed at 340 nm . However in this technique (as in Lynen et al) instead of acetoacetyl-CoA we use AAC[1]; N-ACETYL-S-ACETACETYL-CYSTEAMINE (SIGMA A-5011).

Reagents:

1. AAC 0.1 M
2. Potassium phosphate buffer 0.1M pH 7.0
3. NADH 0.025 M

---

[1]The reason for which AAC was preferred to Acetoacetyl-CoA is the cost of AAC compared to Acetoacetyl-CoA.

Procedure:

1. To a 1 cm cuvette add 1.92 ml of Reagent 2
   0.03 ml of Reagent 3
   and
   0.2 ml of Homogenate solution
   (same for reference and sample cuvettes.)
2. Incubate both cuvettes for 15' at 37°C (±1°C).
3. Add 0.05 ml of Reagent 1 to the sample cell only, and record NADH disappearance with Time (cell holder is kept at 37°C).

N.B.: The background activity due to oxidation of NADH in absence of AAC is compensated for by having the homogenate solution in both cells but AAC only in the sample cell.

NADP-SPECIFIC ISOCITRATE DEHYDROGENASE (T-ICDH)
(E.C. 1.1.1. 42)

Modified from: Lowry, O.H. and J.V. Passonneau.
A Flexible System of Enzymatic Analysis
New York, 1972, pp. 95-96, Academic Press

Principle: $\text{ISOCITRATE} \xrightarrow[\text{T-ICDH}]{\text{NADP} \quad \text{NADPH} + \text{H}^+} \alpha\text{-Ketoglutarate}$

Isocitrate dehydrogenase catalyzes the oxidation of isocitrate to α-Ketoglutarate. The activity of ICDH is measured by recording the amount of NADPH formed per unit of time.

Reagents:

1. D-ISOCITRATE 0.02 M
2. Tris-HCl buffer 0.2 M pH 7.0
3. NADP 0.02 M
4. $MnCl_2$ 0.02 M
5. ALBUMIN 0.02%

Procedure:

1. Stock Solution[1]

   Mix together the following reagents. (vol. is vol/cell/assay)

   2.0 ml of Reagent 2

   0.07 ml of Reagent 3

   0.01 ml of Reagent 4

   0.07 ml of Reagent 1

---

[1] Stock solution is prepared for many (≃ 70) assays and kept cold (<5°C).

1. 0.05 ml of Reagent 5
2. Add 2.2 ml of stock solution (A) to a 1 cm cuvette (both reference and sample).
3. Incubate for 15 min at 37°C (±1°C).
4. Add 0.01 ml of Homogenate solution[1] to the sample cell only.
5. Record rate of NADPH formation at 340 nm against time.

[1] If background activity is observed in absence of isocitrate it must be subtracted from total activity.

HEXOKINASE (HK) (E.C. 2.7.1.1)
(ATP: D-Hexose 6-phosphotransferase)

Modified from: Joshi M.D., and V. Jagannathan
in Methods in Enzymology
Ed. S.P. Colowick and N.O. Kaplan
Vol. IX, p. 371, 1966

Principle: $\text{HEXOSE} + \text{ATP} \xrightarrow{\text{HK}} \text{HEXOSE-6-PHOSPHATE} + \text{ADP}$

Glucose 6-phosphate formed by hexokinase reaction is measured by adding glucose 6-phosphate dehydrogenase and NADP and following NADPH formation. The method minimizes inhibition due to glucose 6-phosphate by oxidizing it to 6-phosphogluconic acid.

Reagents:

1. Glucose 0.15 M
2. $MgCl_2$-$6H_2O$ 0.2 M
3. Tris-HCl buffer 0.2 M, pH 7.0
4. NADP 0.0013 M
5. EDTA 0.0001 M
6. ATP (Na Salt) 0.3 M pH 7.0
7. Glucose 6-phosphate dehydrogenase 2 units/ml, it should have negligible amount of glucose 6-phosphate, NADPase, NADH oxidase, Hexokinase, glucose NADP reductase and 6-phosphogluconic dehydrogenase.

Procedure:

1. Stock solution[1]

   Mix together 0.3 ml (per cuvette/assay) of the reagents 1, 2, 4, 5 and 0.4 ml of reagent 3.

2. Add 1.7 ml of stock solution to a 1 cm cuvette with 0.3 ml of reagent 7
   and 0.1 ml of Homogenate solution (sample cuvette)
   and 0.1 ml of reagent 3 (reference cuvette).

3. Incubate both cuvettes for 15 minutes at 37°C (±1°C).

4. Add 0.1 ml of reagent 6 to both cuvettes and record rate of formation of NADPH at 340 against time. (Cell holder kept at 37°C).

5. If some background activity is measured due to glucose-NADP reductase and the Hexokinase activity of glucose 6-phosphate dehydrogenase correction must be made by measuring NADP reduction in controls without ATP and without Hexokinase respectively.

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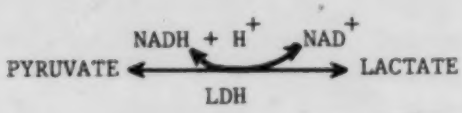
[1] Prepared for about 20 assays and kept cold (<5°C).

N.B. Check for background activities needed to be done and proved to be nonexistant with this technique.

LACTATE DEHYDROGENASE (LDH)
(E.C. 1.1.1. 27)
(L-Lactate: NAD oxidoreductase)

Modified from: Bernstein, L.H. and J. Everse
in Methods in Enzymology
Ed. S.P. Colowick and N.O. Kaplan
Vol. XLI, p. 47, 1975

Principle:



Pyruvate is oxidized to lactate by lactate dehydrogenase and the coenzyme NADH makes the H^+ available to do so.

Reagents:

1. NADH 0.013 M
2. PYRUVATE 0.10 M
3. Potassium Phosphate buffer 0.1 M, pH 7.0

Procedure:

1. To a 1 cm cuvette add 2.9 ml of reagent 3 and 0.005 ml of Homogenate solution.
2. Incubate for 15 min at 37°C (±1°C).
3. Add 0.06 ml of reagent 1, mix thoroughly.
4. Record rate of disappearance of NADH against time at 340.[1]

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[1] Because of the absence of any background activity at this low concentration of enzyme reading can be made against distilled water.

APPENDIX C

RAW DATA

TABLE 10

RAW DATA FOR BODY AND MUSCLE WEIGHTS
OF SEDENTARY AND ANAEROBICALLY TRAINED RATS

GROUPS	BODY WEIGHT (g)	MUSCLE WEIGHT (mg)
C_1	276	1109
	299	1307
	293	1266
	310	1290
	263	1175
	291	1388
	256	1234
	271	1256
C_2	363	1836
	345	1863
	356	1890
	340	1684
	334	1522
	324	1499
	325	1766
	367	1808
C_3	386	1917
	404	2068
	453	2262
	343	1438
	424	1857
	392	1761
	326	1639
	443	1837
	450	1980
E_1	262	1297
	259	1190
	230	1041
	277	1504
	284	1466
	265	1273
	241	990
	251	1322
E_2	339	1546
	340	1672
	353	1696
	337	1445
	351	1787
	304	1182
	302	1588
	344	1635
	376	1831

TABLE 10, cont'd

RAW DATA FOR BODY AND MUSCLE WEIGHTS
OF SEDENTARY AND ANAEROBICALLY TRAINED RATS

GROUPS	BODY WEIGHT (g)	MUSCLE WEIGHT (mg)
T	295	1505
	317	1645
	277	1478
	326	1810
	302	1629
	309	1638
	325	1638
C_{3A}	366	1731
	361	1577
	346	1541

TABLE 11

RAW DATA FOR CALCULATION OF CORRELATION
OF ENZYME ACTIVITY* TO ANAEROBIC PERFORMANCE**

GROUPS	LDH	β-HOADH	ICDH	HK	RUN TIME
E_2	1599	0.33	10.8	0.67	83
	1426	0.33	16.1	0.54	75
	2232	0.27	11.0	0.55	90
	1169	0.40	16.2	0.54	90
	2176	0.38	7.7	0.51	120
	1397	0.36	11.5	0.71	45
	1602	0.32	12.9	0.80	75
	1790	0.34	11.0	0.82	75
	1900	0.26	10.6	0.53	75
T	1233	0.26	18.5	0.92	195
	1487	0.46	21.1	0.79	195
	1279	0.39	10.5	0.65	150
	1382	0.27	10.9	0.95	195
	1311	0.26	15.8	0.92	210
	1454	0.35	11.2	0.56	240
	1640	0.27	8.3	0.96	210

*Activity in μm/min·gram

**Performance: Run time in seconds

TABLE 12

RAW DATA OF ENZYME ACTIVITIES (GASTROCNEMIUS MUSCLE)
OF SEDENTARY AND ANAEROBICALLY TRAINED RATS
(μ moles x min^{-1} x g wet $weight^{-1}$)

GROUPS	LDH	ICDH	HK	β-HOADH
C_1	1176	22.29	0.55	0.55
	1672	15.45	0.51	0.26
	1276	14.29	0.53	0.53
	1029	18.48	0.66	0.53
	1470	7.84	0.52	0.26
	1122	11.21	0.70	0.35
	1123	10.64	0.53	0.33
	1136	17.99	0.90	0.60
C_2	1219	11.26	0.42	0.42
	993	13.49	0.54	0.40
	1256	10.55	0.39	0.26
	1235	10.87	0.54	0.41
	1378	10.19	0.25	0.32
	1231	18.51	0.53	0.46
	1435	10.61	0.53	0.40
	1259	10.34	0.51	0.32
C_3	1171	9.47	0.54	0.27
	1255	12.88	0.26	0.26
	1172	16.24	0.54	0.54
	2144	11.32	0.42	0.28
	1780	8.22	0.41	0.27
	750	5.54	0.41	0.21
	1581	11.02	0.55	0.27
	1310	15.79	0.39	0.39
	1960	16.47	0.27	0.41
E_1	1136	10.50	0.65	0.26
	1320	11.35	0.71	0.42
	1474	21.78	0.95	0.41
	1255	10.08	0.50	0.38
	1548	11.00	0.96	0.34
	1363	10.95	0.82	0.41
	1322	16.29	0.95	0.47
	1286	21.13	0.79	0.66
E_2	1599	10.75	0.67	0.33
	1426	16.14	0.54	0.33
	2232	11.00	0.55	0.27
	1169	16.20	0.54	0.40
	2176	7.73	0.51	0.38

TABLE 12, cont'd

RAW DATA OF ENZYME ACTIVITIES (GASTROCNEMIUS MUSCLE)
OF SEDENTARY AND ANAEROBICALLY TRAINED RATS
(μ moles x min^{-1} x g wet $weight^{-1}$)

GROUPS	LDH	ICDH	HK	β-HOADH
E_2	1397	11.47	0.71	0.36
	1602	12.87	0.80	0.32
	1790	11.03	0.82	0.34
	1900	10.64	0.53	0.26
T	1233	18.54	0.92	0.26
	1487	21.14	0.79	0.46
	1279	10.50	0.65	0.39
	1382	10.86	0.95	0.27
	1311	15.80	0.92	0.27
	1454	11.20	0.56	0.35
	1640	8.26	0.96	0.27
C_{3A}	1481	10.95	0.41	0.41
	1809	7.37	0.37	0.24
	1112	12.84	0.38	0.32

TABLE 13

RAW DATA OF ENZYME RATIOS (GASTROCNEMIUS MUSCLE)
FOR SEDENTARY AND ANAEROBICALLY TRAINED RATS

GROUPS	LDH/ICDH	HK/ICDH	β-HOADH/ICDH	LDH/HK
C_1	52.8	0.025	0.025	2120
	108.2	0.033	0.017	3266
	89.3	0.037	0.037	2392
	55.7	0.036	0.028	1566
	187.6	0.066	0.033	2827
	100.1	0.026	0.031	1609
	105.5	0.050	0.031	2121
	63.1	0.050	0.033	1269
C_2	108.2	0.037	0.037	2900
	73.6	0.040	0.030	1848
	119.1	0.037	0.025	3189
	113.6	0.050	0.037	2283
	135.3	0.025	0.031	5436
	66.5	0.028	0.025	2339
	135.3	0.050	0.037	2717
	121.8	0.050	0.031	2446
C_3	123.7	0.057	0.028	2174
	97.4	0.020	0.020	4895
	72.2	0.033	0.033	2175
	189.4	0.037	0.025	5073
	216.5	0.050	0.033	4350
	135.3	0.075	0.037	1812
	143.4	0.050	0.025	2881
	83.0	0.025	0.025	3334
	119.1	0.017	0.025	7174
E_1	108.2	0.062	0.025	1739
	116.4	0.062	0.037	1870
	67.7	0.044	0.019	1554
	124.5	0.050	0.037	2500
	140.7	0.087	0.031	1615
	124.5	0.075	0.037	1667
	81.2	0.058	0.029	1398
	60.9	0.037	0.031	1631
E_2	148.8	0.062	0.031	2390
	88.4	0.033	0.021	2663
	202.9	0.050	0.025	4077
	72.2	0.033	0.025	2175
	281.4	0.066	0.050	4240
	121.8	0.062	0.031	1958
	124.5	0.062	0.025	2000

TABLE 13, cont'd

RAW DATA OF ENZYME RATIOS (GASTROCNEMIUS MUSCLE)
FOR SEDENTARY AND ANAEROBICALLY TRAINED RATS

GROUPS	LDH/ICDH	HK/ICDH	β-HOADH/ICDH	LDH/HK
E_2	162.4	0.074	0.031	2174
	178.6	0.049	0.025	3588
T	66.4	0.050	0.014	1336
	70.3	0.037	0.022	1885
	121.8	0.062	0.037	1957
	127.2	0.087	0.025	1460
	82.9	0.058	0.017	1429
	129.9	0.050	0.031	2609
	198.4	0.116	0.033	1708
C_{3A}	135.3	0.037	0.037	3624
	245.3	0.050	0.033	4930
	86.5	0.030	0.025	2899

APPENDIX D

SUMMARY OF ANALYSES OF VARIANCE AND NEWMAN KEULS TESTS

SUMMARY OF THE ANALYSIS OF VARIANCE FOR BODY WEIGHTS
OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	6	0.113	18915.000	28.660
ERROR	45	0.297	659.870	

$P < 0.01$

HOMOGENEITY OF VARIANCE CHI SQ = 15.103 $P < 0.05$

SUMMARY OF THE ANALYSIS OF VARIANCE FOR MUSCLE WEIGHT OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	6	0.258	430245.310	14.890
ERROR	45	0.130	28895.640	

$P < 0.01$

HOMOGENEITY OF VARIANCE CHI SQ = 9.446 $P > 0.05$

NEWMAN-KEULS COMPARISON BETWEEN ORDERED MEANS FOR MUSCLE WEIGHT (GASTROC.) OF SEDENTARY, EXERCISED AND TRAINED RATS

		C_3	C_2	T	C_{3A}	E_2	E_1	C_1
	MEANS	1862.111	1733.500	1620.428	1616.333	1598.000	1260.350	1253.125
C_1	1253.125	608.986	480.375	367.303	363.208	344.875	7.225	0.0
E_1	1260.350	601.761	473.150	360.079	355.984	337.650	0.0	
E_2	1598.000	264.111	135.500	22.428	18.333	0.0		
C_{3A}	1616.333	245.778	117.167	4.095	0.0			
T	1620.428	241.683	113.072	0.0				
C_2	1733.500	128.611	0.0					
C_3	1862.111	0.0						
	R=	7	6	5	4	3	2	

The Multiplier is 66.56567

SIGNIFICANT DIFFERENCES

GROUPS	C_1	C_2	C_3	C_{3A}	E_1	E_2	T
T	*	-	*	-	*	-	
E_2	*	-	-	-	*		
E_1	-	*	*	*			
C_{3A}	*	-	-				
C_3	*	-					
C_2	*						
C_1							

* $P < 0.05$ - N.S.

SUMMARY OF THE ANALYSIS OF VARIANCE FOR LDH ACTIVITY
IN GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.122	244198.380	3.21
ERROR	43	0.327	76158.130	

$P < .05$

HOMOGENEITY OF VARIANCE CHI SQ = 19.945 $P < 0.01$

SUMMARY OF THE ANALYSIS OF VARIANCE FOR T-ICDH ACTIVITY
IN GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.684	13.68	0.84
ERROR	43	0.698	16.24	

$P > 0.05$

HOMOGENEITY OF VARIANCE CHI SQ = 4.568 $P > 0.05$

SUMMARY OF THE ANALYSIS OF VARIANCE FOR HEXOKINASE ACTIVITY IN GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.106	0.21	12.10
ERROR	43	0.755	0.02	

$P < 0.01$

HOMOGENEITY OF VARIANCE CHI SQ = 2.504 $P > 0.05$

NEWMAN-KEULS COMPARISON BETWEEN ORDERED MEANS FOR HEXOKINASE ACTIVITY IN GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

		T	E_1	E_2	C_1	C_2	C_3
	MEANS	0.821	0.790	0.630	0.613	0.464	0.422
C_3	0.422	0.399	0.368	0.208	0.191	0.043	0.0
C_2	0.464	0.357	0.326	0.166	0.148	0.0	
E_2	0.630	0.191	0.160	0.0			
E_1	0.790	0.031	0.0				
T	0.821	0.0					
	R=	6	5	4	3	2	

The Multiplier is 0.04653

SIGNIFICANT DIFFERENCES

GROUPS	C_1	C_2	C_3	E_1	E_2	T
T	*	*	*	-	*	
E_2	-	*	*	*		
E_1	*	*	*			
C_3	*	-				
C_2	*					
C_1						

*P < 0.05
- N.S.

SUMMARY OF THE ANALYSIS OF
VARIANCE FOR β-HOADH ACTIVITY

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.889	0.02	1.91
ERROR	43	0.401	0.01	

$P > 0.05$

HOMOGENEITY OF VARIANCE CHI SQ = 10.488 $P > 0.05$

SUMMARY OF THE ANALYSIS OF VARIANCE FOR LDH/T-ICDH RATIO
OF GASTROCNEMIUS MUSCLE OF SEDENTARY,
EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	19495.5	3899.100	1.93
ERROR	43	86999.4	2023.240	

$P > 0.05$

HOMOGENEITY OF VARIANCE CHI SQ = 7.0576 $P > 0.05$

SUMMARY OF THE ANALYSIS OF VARIANCE FOR HK/ICDH RATIO
OF GASTROCNEMIUS MUSCLE OF SEDENTARY,
EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.005	0.00	3.02
ERROR	43	0.001	0.00	

$P < 0.05$

HOMOGENEITY OF VARIANCE CHI SQ = 7.197 $P > 0.05$

NEWMAN-KEULS COMPARISON BETWEEN ORDERED MEANS FOR HK/T-ICDH RATIO OF GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

		T	E_1	E_2	C_1	C_3	C_2
	MEANS	0.066	0.059	0.055	0.045	0.040	0.040
C_2	0.040	0.026	0.020	0.015	0.005	0.001	0.0
C_3	0.040	0.025	0.019	0.015	0.005	0.0	
C_1	0.055	0.021	0.014	0.010	0.0		
E_2	0.055	0.011	0.005	0.0			
E_1	0.059	0.006	0.0				
T	0.066	0.0					
	R=	6	5	4	3	2	

The Multiplier is 0.00610

SIGNIFICANT DIFFERENCES

GROUPS	C_1	C_2	C_3	E_1	E_2	T
T	-	*	*	-	-	
E_2	-	-	-	-		
E_1	-	-	-			
C_3	-	-				
C_2	-					
C_1						

*P < 0.05
- N.S.

SUMMARY OF THE ANALYSIS OF VARIANCE FOR β-HOADH/ICDH RATIO IN GASTROCNEMIUS MUSCLE OF SEDENTARY, EXERCISED AND TRAINED RATS

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE	F
GROUPS	5	0.0002	0.00	0.75
ERROR	43	0.0002	0.00	

$P > 0.05$

HOMOGENEITY OF VARIANCE CHI SQ = 3.056 $P > 0.05$

APPENDIX E

LABORATORY CHOW CONTENT

PURINA CHOW:

Crude protein not less than . . .	23.0%
Crude fat not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	9.0%

Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, ground extruded corn, ground oat groats, soybean meal, dehydrated alfalfa meal, can molasses, animal fat preserved with BHA, vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewers' dried yeast, thiamin, niacin, vitamin A supplement, D activated plant sterol, vitamin E supplement, calcium carbonate, dicalcium phosphate, iodized salt, iron sulfate, iron oxide, magnesium oxide, cobalt carbonate, copper oxide, zinc oxide.

APPENDIX F

TRAINING PROGRESSION UP TO 80 METERS PER MINUTE
(2 WEEKS OF ORIENTATION)

TABLE 14

TRAINING PROGRESSION UP TO 80 METERS PER MINUTE[1]
(2 WEEKS OF ORIENTATION)

DAY	SPEED m/min	TIME ON min:sec	TIME OFF min:sec	NO. OF INTERVALS	TIME OF DAY
1	18	3:0	2:0	6[2]	A.M.
	-	-	-	-	
2	-	-	-	-	
	25	6:0	3:0	7[2]	P.M.
3	35	4:0	4:0	4[2]	P.M.
4	45	2:0	3:0	4[2]	A.M.
	45	2:0	3:0	4[2]	P.M.
7	45	3x(1'on 2'off)	3:0	4[2]	A.M.
	50	3x(1'on 2'off)	3:0	5[2]	P.M.
8	60	3x(30"on 2'off)	3:0	5[2]	A.M.
	60	3x(30"on 2'off)	3:0	4[2]	P.M.
10	65	5x(15"on 20"off)	3:00	2	A.M.
	70	5x(15"on 20"off)	3:00	4	P.M.
11	75	3x(15"on 20"off)	2:00	2	P.M.
14	75	5x(15"on 20"off)	2:00	2	A.M.
	80	0:15	0:30	6	P.M.
15	80	0:15	0:25	7	A.M.
	80	0:15	0:25	6	P.M.
16	80	0:15	0:20	5	A.M.
17	80	0:15	0:20	6	A.M.
	80	0:15	0:20	7	P.M.

[2] one interval = 15 sec. on, 20 sec. off

[1] at 30% incline